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Quantification of volatile compounds (oxidative and olfactory) in meat products and impact of seaweed addition on the sensory and volatile profiles of processed meat products

Thesis presented by

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for the degree of

Doctor of Philosophy

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, at either University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism and intellectual property.

Signature:



Elena Garicano Vilar **Date:** 3 October 2022

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Preface

This work has been written as part of the graduation requirements for the Degree of Doctorate in Food Science and Technology at University College Cork, Ireland. The research and writing period of this thesis has lasted from January 2018 to September 2022.

After four and a half intense years, today is the day. It is time to thank all those people who have helped and supported me during this process.

I would like to thank Teagasc, University College Cork, the Department of Agriculture Food and Marine (DAFM), and the Walsh Scholar Program for giving me the opportunity to carry out my research at Teagasc Food Research Centre, Moorepark, and to complete this PhD.

I would like to thank my supervisors, Prof. Kieran Kilcawley, Dr. Maurice O'Sullivan and Prof. Joe Kerry for their excellent guidance throughout the completion of my work. To Kieran, special thanks for his tutoring, knowledge and for praising my work. I would also like to thank David Mannion, without whom I would not have thrived in the lab. All his wisdom and skill always fascinated me. Another thank you to all in the Flavour Chemistry lab for their time and collaboration.

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"*Lucha por ser feliz, trabaja duro y diviértete cuanto puedas*" – Lastly, I would like to express gratitude to my family. Thank you for being proud of me and my progress in life. Thank you for wishing me the best and trusting that I will achieve everything I wish for. Thank you for admiring my strength and ability to overcome. I

like to think that you have something to do with all this. Your wise advice and encouragement have been of great help to me, always. To my nephew, for making me smile every day.

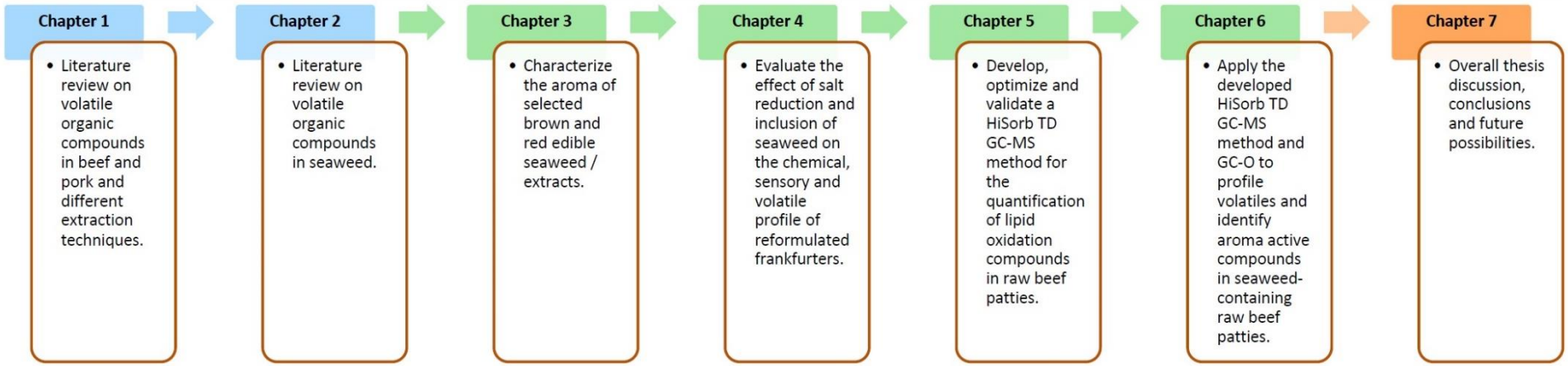
It has been a period of intense learning, not only in the scientific field, but also on a personal level. Carrying out this work has had a great impact on me. I hope you all enjoy reading the result.

E. Garicano Vilar

Finland, 1st October 2022.

Thesis objectives

The overall thesis objective was to optimise processed meats (frankfurters, beef patties) formulations using a clean label approach through salt reduction and the incorporation of edible seaweed, and to understand the influence of matrix changes on the character aroma compounds and sensory perception, and lipid oxidation and shelf life of the developed products. The objectives of individual chapters are presented in the figure below.



Abstract

Now more than ever consumers demand healthier food options, including meat products, with 'clean-label', sustainable and natural ingredients without compromising their sensory experience; hence a greater need for innovative concepts and approaches towards new product development is required. In this regard, seaweeds compose a credible source of functional ingredients with bioactive properties.

There is a positive association between processed meat consumption and the risk of chronic diseases, such as cardiovascular disease, certain cancers or type II diabetes, due to their high levels of saturated fats and sodium. Processed meat refers to meat that has been transformed through salting, curing, fermentation, smoking, or other processes to enhance flavour or improve preservation (i.e., frankfurters, ham, and canned meat). The optimization of processed meat products through the reduction of fat and the partial replacement of salt could potentially offset the risk. However, the influence of matrix changes on the character aroma compounds and sensory perception of the developed products could adversely affect consumer acceptability. Therefore, this research aims to characterize the volatile organic compounds (VOC) and aroma profiles of processed meat products and seaweed, and to study seaweed-based processed meat products in terms of shelf life and consumer perception.

This thesis has demonstrated the feasibility of generating valuable VOC and aroma profiles of processed meat products and seaweed using sophisticated extraction, separation, and identification gas chromatography mass spectrometry techniques (GC-MS); and of developing a novel method using headspace high capacity sorptive (HiSorb) extraction for quantification of target lipid oxidation compounds. This in-depth approach provides information on the sensory characteristics and shelf life of the processed meat products (frankfurters and beef patties), following reformulation and/or storage. The work in this thesis primarily employed HiSorb and thermal desorption (TD) GC-MS (HiSorb TD GC-MS) with polydimethylsiloxane (PDMS) probes and headspace solid phase microextraction GC-

MS (HS-SPME GC-MS) for VOC profiling, but also the use of gas-chromatography olfactrometry (GC-O) applications.

Chapter 1 provides an updated insight into the main extraction/concentration techniques that are available for the determination of the main odour-active volatiles in beef and pork. Aroma compounds are challenging to extract, concentrate, separate, identify and quantify due to the complexity of meat. The review highlights the potential pros and cons of the main approaches used to date but also, how advances in extraction, chromatography and olfactometry helps elucidate more key aroma compounds. However, many VOC have been identified in beef and pork to date. It is the use of GC-MS and olfactometry methodologies that enable us to increase our knowledge of the importance of the key volatiles impacting on consumer perception, either positively or negatively.

The review in **chapter 2** provided insights into the volatile analyses of six commercially important brown and red seaweed species used in the food industry and highlighted the need for more information regarding VOC in edible macroalgae and to identify those most likely to impact sensory perception. This is as important for those VOC that positively or negatively contribute to sensory perception. Such information could be used to aid new product development or widen applications of these seaweeds in the food sector, one of which is in meat product formulation. In that regard, **chapter 3** investigated the volatile profiles of dried brown (*Himanthalia elongata*, *Undaria pinnatifida*, *Alaria esculenta*) and red (*Porphyra umbilicalis*, *Palmaria palmata*) seaweeds, and a brown seaweed extract (fucoxanthin) from *Laminaria japonica*, some of which had not previously been described. A chemometric approach was used to collate data from GC-MS, direct sensory aroma evaluation, and GC-O to obtain a better understanding of their volatile profile and sensory perception. Once the volatile profiles of the seaweed were characterized, **chapter 4** investigated the impact of the inclusion of four selected seaweeds (1 % w/w) in reformulated frankfurters in which salt addition was reduced by 50 % compared to a control. The overall acceptability of reformulated frankfurters containing seaweed was greatly influenced by the type of added seaweed (also linked to dosage), but overall, the addition of seaweed enabled salt reduction and thus improved the nutritional quality. Further processing of seaweed prior to addition,

optimization of dose rates, combinations of seaweeds and/or highlighting potential nutritional benefits will be necessary before such products are accepted by consumers unfamiliar with seaweeds in their diet.

Lipids may cause both desirable and undesirable flavours and aromas in meat. However, progressive lipid oxidation adversely affects meat quality. Sophisticated techniques such as GC-MS can be used to identify and quantify individual VOC associated with lipid oxidation. Hence, the aim in **chapter 5** was to develop a GC-MS method using HiSorb extraction for untargeted volatile profiling and to quantify targeted LOC in raw beef patties over refrigerated storage. Using this approach to determine the concentrations of VOC associated with lipid oxidation that adversely impact sensory perception provided insights into the production parameters that could maximise the oxidative shelf life stability and quality of meat products.

It is essential to expand the knowledge on how to protect foods against lipid oxidation. Synthetic antioxidants have been used for many years to retard lipid oxidation in foods. However, there is growing interest in replacing synthetic antioxidants with natural ingredients. Seaweeds are not only a valuable source of bioactive compounds and a good salt content replacer, but also exhibit extraordinary antioxidant potential which can be harnessed for a broad variety of food applications. Therefore, the validated HiSorb GC-MS method outlined in **chapter 5** was applied to profile the aroma of raw beef patties reformulated with seaweed, packed in MAP, and stored under refrigeration conditions in **chapter 6**. The method was combined with GC-O to detect VOC changes that could cause off-flavours in the product over time, along with changes in colour and TBARS values indicative of lipid oxidation. The antioxidant activity of the seaweed was also examined in an attempt to evaluate their effect against product deterioration. The antioxidant activity present in seaweed, attributable to polyphenols, flavonoids and other antioxidants, opens new possibilities of application of these in fresh meat products. Their use could potentially increase shelf life of food products due to their antioxidant capacity, and replace artificial food additives.

Dissemination

Peer-reviewed articles

1. Garicano Vilar E, O'Grady MN, Mannion DT, O'Sullivan MG, Kerry JP, Kilcawley KN. Optimization of a high-capacity sorptive extraction gas chromatrography-mass spectrometry method for untargeted volatile profiling and for the quantification of targeted lipid oxidation volatile compounds in raw beef patties. *Peer reviewed journal*. 2022; [*under review*].
2. Garicano Vilar E, O'Sullivan MG, Kerry JP, Kilcawley KN. Volatile organic compounds in beef and pork by gas-chromatography mass-spectrometry: A review. *Sep Sci Plus*. 2022; 1-31. DOI: 10.1002/sscp.202200033.
3. Garicano Vilar E, O'Sullivan MG, Kerry JP, Kilcawley KN. Volatile compounds of six species of edible seaweed: A review. Algal Research. 2020; 45: 101740. DOI: 10.1016/j.algal.2019.101740.
4. Garicano Vilar E, O'Sullivan MG, Kerry JP, Kilcawley KN. A chemometric approach to characterise the aroma of selected brown and red edible seaweeds/extracts. J Sci Food Agric. 2021; 101(3): 1228-1238. DOI: 10.1002/jsfa.10735.
5. Garicano Vilar E, Ouyang H, O'Sullivan MG, Kerry JP, Hamill RM, O'Grady MN, Mohammed HO, Kilcawley KN. Effect of salt reduction and inclusion of 1 % edible seaweeds on the chemical, sensory and volatile component profile of reformulated frankfurters. Meat Science. 2020; 161: 108001. DOI: 10.1016/j.meatsci.2019.108001.

Conferences

1. Oral – Effect of salt replacement with 1 % edible seaweeds and fat reduction on the chemical, sensory and volatile flavour characteristics of reformulated frankfurters. 2nd Food Chemistry Conference, Sevilla, Spain, 17-19th September 2019.

2. Poster – Effect of salt and fat replacement by seaweed on the sensory and volatile component profile of frankfurters. 47th Annual Food Science and Technology Conference, Cork, Ireland, 6-7th December 2018.
3. Poster – Volatile component and odour profiles of three brown and two red edible species of seaweeds and a brown seaweed extract. 48th Annual Food Science and Technology Conference, Limerick, Ireland, 16th December 2019.
4. Poster – Optimization of a high-capacity sorptive extraction (Hi-Sorb) method coupled to gas chromatrography-mass spectrometry for the determination of lipid oxidation volatile compounds in beef meat. 66th International Congress of Meat Science and Technology and 73rd Reciprocal Meat Conference, Florida, USA, 3-6th August 2020.
5. Poster – Chemometric analysis of volatile flavour sensory characteristics of reduced salt reformulated frankfurters containing 1 % w/w edible seaweeds. 9th European Conference on Sensory and Consumer Research, Rotterdam, The Netherlands, 6-9th September 2020.
6. Poster – Development of a high-capacity sorptive extraction (Hi-Sorb) gas chromatrography-mass spectrometry method for the quantification of volatiles associated with lipid oxidation in raw beef. 49th Annual Food Science and Technology Conference, Dublin, Ireland, 15th December 2020.
7. Poster – Optimization of a high-capacity sorptive extraction (Hi-Sorb) GC-MS method for the determination of lipid oxidation volatile compounds in beef. Irish Mass Spectrometry Society Conference, Dublin, Ireland, 11-12th May 2021.

List of abbreviations

ACC	Aerobic colony count
AMDIS	Mass spectral deconvolution and identification system
Amu	Atomic mass units
ANOVA	Analysis of variance
APLSR	ANOVA partial least squares regression
aW	Water content activity
Ca	Calcium
CAR/PDMS	Carboxen™/polydimethylsiloxane
CCRD	Central composite rotatable design
CFU	Colony-forming unit
CW/DVB	Carbowax-Divinylbenzene
CW/TPR	Carbowax-Template resin
DE	Distillation extraction
DHS	Dynamic headspace extraction
DI	Direct immersion
DI-SPME	Direct immersion solid-phase microextraction
DPPH	2,2-Diphenyl-1-picryl-hydrazyl-hydrate
DVB/CAR/PDMS	Divinylbenzene/carboxen™/polydimethylsiloxane
DVB/CWR/PDMS	Divinylbenzene/carbon wide range/ polydimethylsiloxane
ECC	*Escherichia coli* count
ECD	Electron capture detector
FID	Flame ionization detector
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GC	Gas chromatography
GCxGC	Two-dimensional gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GC-O	Gas chromatography-olfactometry

HiSorb	High capacity sorptive extraction
HS	Headspace
HS-SPME	Headspace solid-phase microextraction
IS	Internal standard
ISO	International Organization for Standardization
K	Potassium
LAB	Lactic acid bacteria
LLE	Liquid-liquid extraction
LOC	Lipid oxidation compound
LOD	Limits of detection
LOQ	Limits of quantification
LRI	Linear retention indices
MAP	Modified atmosphere packaging
MDA	Malondialdehyde
Mg	Magnesium
MHSSE	Multiple headspace sorptive extraction
MRD	Maximum recovery diluent
MS	Mass spectrometry / spectrometer
MSD	Mass selective detector
m/z	Mass-to-charge ratio
N	Nitrogen or Normality
Na	Sodium
NaCl	Sodium chloride
NANS	National adult nutrition survey
NBS	National Bureau of Standards
NIST	National Institute of Standards and Technology
OAV	Odour activity value
PA	Polyacrylate
PA/PE	Polyamide/polyethylene
PEG	Polyethylene glycol
PCA	Principal component analysis

PDMS	Polydimethylsiloxane
PDMS/CWR	Polydimethylsiloxane/Carbon wide range
PDMS/DVB	Polydimethylsiloxane/divinylbenzene
PLSR	Partial least squares regression
ppb	Parts-per-billion
ppt	Parts-per-trillion
PSE	Pressurized solvent extraction
PTFE	Polytetrafluoroethylene
P&T	Purge & trap
Q	Single quadrupole
RDA	Ranking descriptive analysis
RH	Relative humidity
RSA	Radical scavenging activity
RSD	Relative standard deviation
RSM	Response surface methodology
SAFE	Solvent assisted flavour evaporation
SBSE	Stir bar sorptive extraction
SD	Standard deviation
SDE	Simultaneous distillation extraction
SFE	Supercritical fluid extraction
SHS	Static headspace extraction
SPDE	Solid-phase dynamic extraction
SPME	Solid-phase microextraction
SPSS	Statistical package for the social sciences
STP	Standard temperature and pressure
SVOC	Semi volatile organic compounds
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TD	Thermal desorption

TIC	Total ion count
TID	Thermal ionization detector
TOF	Time-of-flight
TPA	Texture profile analysis
TPC	Total phenol content
TQ	Triple-quadrupole
TVC	Total viable count
VOC	Volatile organic compounds

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Chapter 1

Chapter 1. Literature review: Volatile organic compounds in beef and pork by gas-chromatography mass-spectrometry

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1.1. Abstract

The aroma of meat is an integral part of flavour and therefore an essential element for consumer acceptance. Aroma compounds are challenging to extract, concentrate, separate, identify and quantify due to the complexity of meat. A wide range of volatile compounds of different properties (molecular weight, polarity, vapour pressure) are present in raw meat at varying concentrations, and further exacerbated post cooking due to the creation of additional volatiles through mainly Maillard reactions and lipid oxidation reactions. The volatiles identified in cooked meats include hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, lactones, ethers, heterocyclic constituents and sulphur-/halogen-containing components. From published research, some of the key volatiles influencing cooked beef/pork flavour include hexanal, octanal, nonanal, 1-nonanal, (E,E)-2,4-decadienal, methional, 3-methyl-1-butanol, methanethiol, 2-furfurylthiol, 2-metyl-3-furanthiol, 3-mercapto-2-pentanone, 4-hydroxy-2,5-dimethyl-3-(2H)-furanone, 1-penten-3-one, 2-pentyl-furan, N-morpholinomethyl-isopropyl-sulphide and methyl butyrate. Gas chromatography-mass spectrometry (GC-MS) is the method of choice for their separation and identification. The technical options available have never been greater to elucidate these key volatiles impacting sensory perception. This review presents a summary of the main extraction/concentration techniques, highlighting their potential pros and cons, how advances in two-dimensional gas chromatography may help elucidate more key aroma compounds, and outlines the benefits of olfactometry to determine the main odour active volatiles in beef and pork.

1.2. Introduction

Meat is a complex, heterogeneous mixture composed of a multitude of different volatile compounds that contribute to its overall sensory perception (Merkle et al., 2015). Flavour, conformed by taste and aroma, is one of the main factors that instigates consumer acceptance of foods (Maughan et al., 2012). Flavour is the result of the combination of taste compounds registered on the tongue and volatile compounds moved collectively throughout the mouth, sinus and nasal cavities (orthonasal- and retronasal-olfaction) to the odour epithelium in the nose (Aaslyng and Meinert, 2017). Although the basis for meat flavour, such as beef and pork, is established in the primary production phase through choice of breed and feed, it is also influenced by the slaughtering process, and the aging and cooking processes of the meat (Aaslyng and Meinert, 2017).

Beef and pork flavour typically comprises of hundreds of volatile organic compounds (VOC), with trace-level analytes often having the greatest effect on perceived aroma because the odour activity of the individual VOC can be more important than their concentration (Thermal Desorption Solutions, 2019). The analysis of VOC firstly requires their extraction and concentration (Merkle et al., 2015), followed by separation typically undertaken using gas chromatography (GC), and finally identification and possible quantification using one or more detection systems. Due to the wide variety of potential VOC, differing in polarity, volatility, vapour pressure and molecular weight, a wide range of different extraction techniques exist. It is also beneficial to use the human nose as a detector due to its sensitivity, but also as panellist(s) can provide specific aromatic descriptors for volatiles, and thus potentially confer additional information in relation to its perceived intensity, and thus identify those likely contributing most to consumer perception. Volatile concentration is required for olfactometry analysis as extraction and separation alone dilute the odour profile making it very difficult, if not impossible to discern individual VOC (although this can be product specific). Previous studies have identified that the real volatile fraction that reaches the human receptors when a sample is sniffed is better represented by volatiles obtained by dynamic headspace

(DHS), purge-and-trap (P&T), headspace solid phase microextraction (HS-SPME) techniques (Narváez Rivas et al., 2012), or thermal desorption (TD). However, no single extraction method or approach is likely to provide a complete volatile profile of a sample, due to inherent bias associated with the extraction and separation techniques of choice (differences in chemistry of the phases, capacity, etc.).

The analysis technique should be selected based on whether the aim is to target some specific volatiles as a targeted approach, or as an untargeted approach in an attempt to obtain as much of the representative volatile profile as possible. The following factors ought to be also taken into consideration: (a) the sample; the composition of the sample matrix (particular attention to the moisture content and pH), the presence of interfering compounds (especially fat and protein), the amount of sample that requires analysis, the homogeneity of the sample and its availability (all of which should influence the amount of sample required), (b) the analytes of interest; the potential concentration and range present, their thermal stability, the potential range of their boiling points, and volatility, and (c) the extraction methods and equipment availability, cost, time, accuracy, precision and reproducibility (Manura and Overton, 1999).

The aim of this review is to provide an overview of the sample preparation techniques used to access VOC in beef and pork by GC-mass spectrometry (MS) and GC-olfactometry (GC-O), to highlight potential advantages and disadvantages of specific extraction techniques, and to describe the main volatiles contributing to the characteristic flavour of beef and pork to date.

A data search using the major databases (PubMed, ScienceDirect, Springerlink, ResearchGate) with the keywords “dynamic headspace”, “static headspace”, “purge-and-trap”, “thermal desorption”, “volatile organic compounds”, “gas chromatography”, “mass spectrometry”, “meat”, “beef”, “pork”, “flavour” was carried out to identify the relevant original articles for this review. The research highlighted that there is a disparity of information on sample preparation techniques for VOC analysis by GC-MS and therefore this timely review provides a relevant up to date summary of the four most widely used extraction techniques for meat products, DHS, static headspace (SHS), HS-SPME, and TD; and includes novel analytical approaches such as two-dimensional gas chromatography (GCxGC) which

are becoming more widespread due to advances in non-cryogenic flow control and software.

1.3. Development of meat flavour

In summary, meat flavour development is primarily dependent on two factors: reactant mixture or intrinsic factors (i.e. chemical composition and pH) and reaction conditions or extrinsic factors (i.e. thermal kinetics as a result of varied time and temperature exposure). Flavour development, therefore, is highly dependent on the reactant mixture (precursor compounds of flavour) and reaction conditions (cooking), and is described as a complex system of products and interactions. Many VOC are the final products of cooked meat flavour development and therefore their detection presents an opportunity to better understand meat flavour development and how chemical flavour components relate to perceived flavour.

The final composition of VOC in beef and pork products is determined by breed, gender, diet and age of an animal; slaughter process; duration and condition of meat storage; type of muscle; preparation of meat, and type of additives applied as well as condition of heat treatment (cooking, roasting, smoking) and, any technological processes undertaken (Kosowska et al., 2017a). Among the different factors which influence the meat flavour, animal diet is the most important, especially its lipid content, as it is the main source of aromatic volatile compounds (Karabagias, 2018). The flavour of beef and pork is complex as it depends on the interaction of volatile (aroma) and non-volatile (taste) compounds (Grabež et al., 2019). Raw meat, in spite of its weak-aroma and blood-like taste, constitutes a rich matrix of non-volatile precursors (amino acids, peptides, reducing sugars, vitamins, nucleotides and fatty acids) and a diverse pool of VOC, some of which are responsible for its flavour (Aaslyng and Meinert, 2017). Beef and pork develop its aroma characteristics during cooking as a result of interaction of precursors derived from lean and fat components (Van et al., 2012). This occurs *via* four pathways including (a) Maillard reaction of amino acids or peptides with reducing sugars, (b) lipid oxidation, (c) interaction between Maillard reaction products with lipid-oxidized products and (d) thiamine

(vitamin B1) degradation. Maillard reaction products come from high temperature rapid cooking whereas lipid products are generated from low temperature slow cooking (Kerth and Miller, 2015). Even though many different VOC from a range of chemical classes are present, it is estimated that only a small portion (<3 %) are capable of imparting odours to the food (Dunkel et al., 2014), as they need to be at a concentration above their odour thresholds to be perceived. As the odour thresholds of VOC vary markedly and are impacted mainly by product composition, a less abundant VOC can have a greater aromatic impact than others present at significantly higher concentrations.

1.3.1. Maillard reaction

The Maillard reaction is a redox reaction between the amine group of an amino acid and the carbonyl group of a reducing sugar. A cascade of reactions, including (1) sugar-amine condensation, the Amadori rearrangement, (2) sugar breakdown and dehydration, Strecker degradation, and (3) aldol condensation, aldehyde-amine condensation and formation of heterocylic nitrogen compounds via Schiff bases pathways, create a number of VOC that produce desirable flavours in cooked meats (Bertrand et al., 2018; Diez-Simon et al., 2019) (Fig. 1.1). If the initial reducing sugar is an aldose monosaccharide that has an aldehyde group (ribose or glucose), it forms an aldosylamine-N-substituted, which is rearranged to form an intermediary Amadori. If the reducing sugar is a ketose monosaccharide that has a ketone group (ribulose or fructose), Heyns rearrangement of ketosylamine-N-substituted and the corresponding Heyns intermediary are produced (Resconi et al., 2013). Next, the Amadori/Heyns products are degraded, which leads to dehydrations and deaminations that produce carbonyl compounds such as deoxysones and deoxyreductones (Resconi et al., 2013). Then, primary compounds such as furfural, furanone derivatives, hydroxyketones, and dicarbonyl compounds are created. More complex products derived from other amines, amino acids, aldehydes, hydrogen sulphide, and ammonia are also formed. The final stage of the Maillard reaction, the Strecker degradation is the most important step for flavour formation, as it provides

routes by which nitrogen and sulphur can be introduced into heterocyclic compounds (Sun et al., 2022). Strecker degradation is initiated by the reaction between carbonyl compounds and an amino acid forming two important intermediate compounds, α-aminoketones or aminoalcohols and an aldehyde. The Strecker aldehyde itself contributes to the flavour of the meat (Kanokruangrong et al., 2019) and the α-aminoketones are key precursors for heterocyclic compounds, such as pyrazines, pyrroles, oxazoles, thiophenes and thiazoles, and furans, all of which are also important classes of aroma compounds in meat (Kerth and Miller, 2015). The amino acid cysteine has long been associated with food flavour since its decomposition via Strecker degradation leads to numerous low aroma threshold sulphur VOC (hydrogen sulphide, ammonia and acetaldehyde), whereas methionine will yield methional, 2-propenal, methanethiol and dimethyl disulphide (Kanokruangrong et al., 2019; Resconi et al., 2013). Hydrogen sulphide *per se* and its reaction products with aldehydes, furfural and furanones have also been proven to be a diverse source of flavour compounds in meat products (Rizzi, 1999). Some of these compounds, together with carbonyl compounds produced in the Maillard reaction, provide intermediates for reactions giving rise to important aroma compounds (Kanokruangrong et al., 2019). The reaction also leads to the formation of melanoidins, brown and high molecular weight polymers, from the condensation of cyclic compounds (Bertrand et al., 2018). The myriad of compounds derived from the Maillard reaction are responsible for the flavours generally described as roasted, browned, meaty and caramelized (Kerth and Miller, 2015) and are key aromatic volatiles influencing sensory perception.

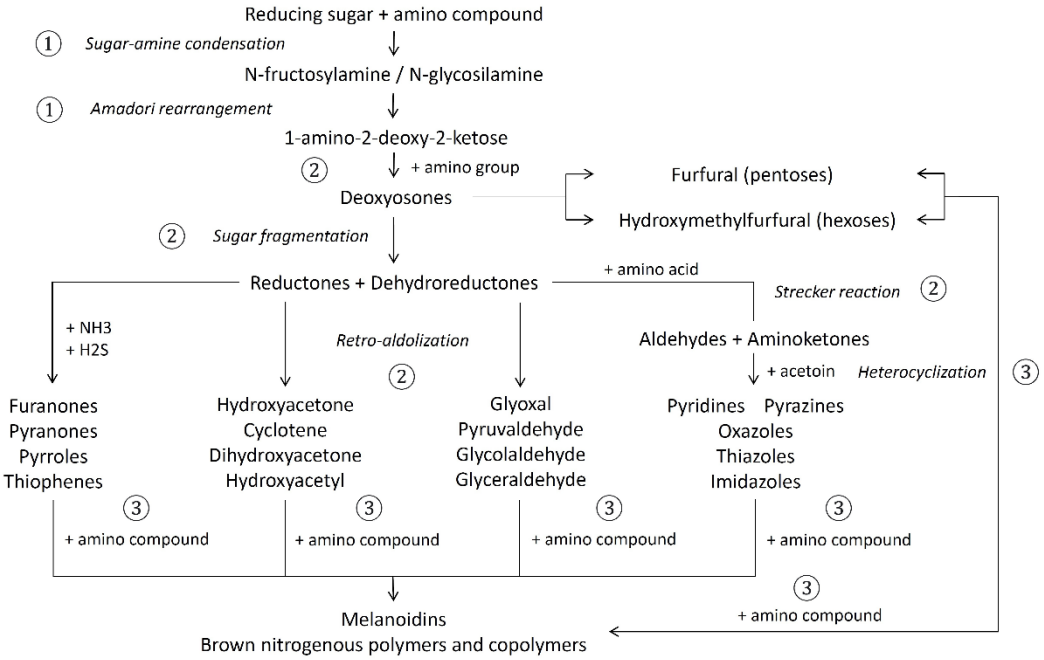


Figure 1.1. Schematic overview of the Maillard reaction showing the main-end products contributing to flavour (adapted from (Sun et al., 2022)).

1.3.2. Lipid oxidation

Lipid oxidation is a major cause of meat quality deterioration during storage, but low levels of lipid oxidation can enhance cooked meat flavour (Kanokruangrong et al., 2019). Fatty acids and lipids contribute greatly to the generation of aromatic VOC that are characteristic of cooked meat (Kanokruangrong et al., 2019). Therefore, both fat content and the fatty acid profile are critical to the formation of many volatiles in cooked meat (Mottram and Edwards, 1983). These are influenced by diet, which highlights the potential of diet in relation to their creation and the sensory perception of cooked meats. Zhao et al. (Zhao et al., 2018) suggested that the fatty acid composition is unique to each species, therefore species flavour must be associated with lipid degradation during cooking (Kosowska et al., 2017). Arshad et al. (Arshad et al., 2018) also postulated that the lean tissue is responsible for the 'meaty' flavour found in all species. However, the phospholipids in the cell membrane of the lean tissue, even though a small fraction of the total lipid content in beef, are extremely important to the formation of volatile flavour components

(Estévez et al., 2003). Intramuscular fat and adipose tissues are now thought to be the main source of volatile flavour components in meat. This fat and adipose tissue contain different proportions of saturated and unsaturated fatty acids, which when degraded and oxidized provide a prolific number of VOC (Mottram and Edwards, 1983). On the contrary, high levels of polyunsaturated fatty acid can cause undesirable flavours as they are more susceptible to oxidation and also inhibit the production of some Maillard reaction products (Mottram and Edwards, 1983). Examples of VOC derived from lipid degradation in cooked meat include aldehydes, alcohols, ketones, esters, carboxylic acids and aliphatic hydrocarbons (Kanokruangrong et al., 2019), all of which can give rise to 'fatty' aromas in cooked meat (Van et al., 2012).

1.3.3. Interactions between Maillard reaction products and lipid-oxidized products

Maillard reaction and lipid oxidation should be considered simultaneously to fully understand the reactions involved and the products formed in complex matrices involving lipids, amino acids and carbohydrates. Maillard reaction products are capable of promoting and reducing lipid oxidation. For example, Amadori products have been shown to increase phospholipid oxidation, and non-enzymatically browned proteins can reduce lipid oxidation. On the other hand, lipid oxidation products can modify the Maillard reaction by promoting or preventing the reaction, or by reacting with some intermediates and producing compounds different from those commonly formed in the absence of lipids. One example of this is the development of aroma in cooked meat, where the presence of lipids is essential for the development of pleasant flavours (Zamora and Hidalgo, 2011). VOC that can be considered as products of Maillard/ lipid interactions have been identified in studies with model systems (Elmore et al., 2002; Xu et al., 2011). The majority of these are heterocyclic compounds containing one or more atoms of nitrogen or sulphur and are characterized by the presence of a long-chain alkyl substituent with four or more carbon atoms (Kosowska et al., 2017). Some of the compounds that are either

derived or could be derived from interactions of Maillard reactions and lipids include alkanethiols (1-heptanethiol, 1-octanethiol), thiophenes (2-butyl/pentyl/hexyl/heptylthiopene), pyridines (2-pentylpyridine), and some alkylsulfides, alkylamines, pyrroles, and thiazoles (Whitfield and Mottram, 1992).

1.3.4. Thiamin degradation

Thiamin degradation is an important flavour generation pathway in cooked meat. Thiamin is susceptible to thermal degradation and produces a multitude of flavour compounds (Resconi et al., 2013), most of them contain one or more sulphur and/ or nitrogen atoms (Van et al., 2012). The primary products of thermally-degraded thiamin include 4-methyl-5-(2-hydroxyethyl)thiazole, which subsequently degrades to form other thiazoles, and 5-hydroxy-3-mercaptopentan-2-one. These can be further degraded to other sulphur-containing compounds, such as thiophenes and specific furans (van der Lide et al., 1979). Some significant thiamin thermal degradation products are 2-methyl-3-furanthiol, bis(2-methyl-3-furyl) disulphide, 3-thiophenethiol, 2-formyl-5-methylthiophene and 2-methyl-3-(methyldithio) furan, all of which produce intense meaty aromas (Dreher et al., 2003). Some of these VOC derived from thiamin degradation can also be produced from the Maillard reaction between cysteine and ribose, the Strecker reactions of sulphur amino acids and their interactions (Resconi et al., 2013).

1.4. Specificity of beef and pork flavour compounds

Different classes of VOC contribute their own attributes to the overall meat flavour. The volatiles identified in cooked beef and pork in general include most types of organic compounds: hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, lactones, ethers, heterocyclic constituents (furans, pyridines, pyrroles, oxazoles and oxazolines, thiazoles and thiazolines, and thiophenes) and other sulphur- as well as halogen-containing components. The odour threshold is a very important concept to understand in discussing the potential sensory importance of

any VOC. The odour threshold is the minimum concentration (usually provided in water, a solvent, fat or air) at which the volatile compound can be perceived orthonasally or retronasally, and is typically expressed in either parts per million (ppm) or parts per billion (ppb) (Grosch, 1994). Nitrogen- and sulphur-containing heterocyclic compounds that are derived from Maillard reactions tend to have the lowest odour thresholds. Lipid-derived VOC, such as ketones and alcohols, tend to have higher odour thresholds and therefore a greater concentration is required before these can be perceived (Czerny et al., 2008). The most odour-active compound found in any product to date is 2-methylfuran-3-thiol (meaty aroma) with an odour threshold of 0.00003 μg/kg (Kerscher and Grosch, 2000; Tang et al., 2012).

To date a large number of VOC contributing to the flavour characteristics of cooked beef have been identified (Van et al., 2012). Some of these key flavour compounds include methional (cooked potato aroma), E-2-undecenal (sweet, fruity, fatty aroma), E-2-dodecenal (sweet, fruity, roasty aroma), decanal (sweet fruity, meaty aroma), heptanal (fruity, fatty, green aroma) and 2-methylbutanal (pungent, sweet, roasty aroma) (Specht and Baltes, 1994). Others such as dimethyl sulphide (sweet, fruity, gas aroma), 2-butanone (chemical, burnt, gas aroma), ethyl acetate (caramel, sweet aroma), 3-methylbutanal (meaty, rotten, fatty, aldehyde, pungent, green, apple, malt aroma), 2-heptanone (citrus, grapefruit, floral, fruity, spicy, cinnamon aroma), nonanal (sweet, green, citrus, floral, waxy aroma) and 2,4-nonadienal (fatty, meaty, burnt, chocolate) are key odours in Angus beef (Machiels et al., 2003, 2004); 2-furfurylthiol (roasty aroma), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (roasted almonds, sweet aroma) and 2-methyl-3-furanthiol (meaty, sweet, sulphurous, roast/boiled meat aroma) are important in boiled beef (Kerscher and Grosch, 1997); whilst 2-ethyl-3,5-dimethyl pyrazine (burnt, fragrant, meaty, green aroma) and 2,3-diethyl-5-methylpyrazine (meaty, roasty, fragrant, sweet aroma) are key VOC in roast beef (Rochat et al., 2007). Some of the individual VOC that have been found to be characteristic of the distinct aroma flavours of beef are listed in Table 1.1. As previously mentioned, lipid-derived VOC are important, the majority of which include saturated and unsaturated aldehydes with six to ten carbons. The most common VOC found in cooked beef are hexanal (green, fatty, grassy, sweet, fresh notes) and nonanal (sweet, fatty, green, pungent, sea, citrus, citronella grass, floral,

grassy, waxy notes) which are key products of enzymatic oxidation of unsaturated fatty acids. This is explained by the fact that the primary substrate for these aldehydes, oleic acid (omega-9 monounsaturated fatty acid), is the most abundant fatty acid in beef (Kerth and Miller, 2015).

The VOC in cooked pork have also been documented (Aaslyng and Meinert, 2017). Analysis of VOC in pork meat has revealed nine chemical classes including aldehydes, alcohols, ketones, furans, sulphides, esters, acids, phenols and hydrocarbons, as well as small amounts of other VOC such as alkenes and pyrroles (Chen et al., 2014, 2019). The VOC thought to contribute to the flavour of pork are hexanal (grass), octanal (fresh), 1-nonanal (delicate), (E,E)-2,4-decadienal (broth), 3-methyl-1-butanol (pungent), 1-penten-3-one (onion), 2-pentyl-furan (ham), N-morpholinomethyl-isopropyl-sulphide (meaty) and methyl butyrate (apple-like) (Chen et al., 2019). Seven other odour-active compounds (hexanal (green grass), heptanal (fatty, putty), nonanal (fatty, floral, wax), 1-octen-3-ol (mushroom), 2-pentylfuran (pungent, sweet), 2-ethylfuran (rubber, pungent) and dimethyl disulphide (cooked cabbage)) have also been identified as the main contributors to the integral flavour of boiled pork due to their higher odour activity values (Han et al., 2020).

Table 1.1. Principal flavour VOC most frequently detected in beef, their odour description, extraction technique and polarity of GC column used for analysis.

Compound	CAS no.	Odour description	Extraction technique	Polarity column	Reference
Aldehydes [a]					
Propanal	123-38-6	Caramel, sweet, alcoholic, "cooked", broth, spicy	HS / SPME	P / NP	(Rochat and Chaintreau, 2005; Van Ba et al., 2010)
Butanal	123-72-8	Smoky, fish, amylic, aldehyde	HS / SPME	P / NP	(Rochat and Chaintreau, 2005; Van Ba et al., 2010)
Pentanal	110-62-3	Almond, malt, pungent, acrid	NR / SPME	NR / NP	(Calkins and Hodgen, 2007; Van Ba et al., 2010)
Hexanal	66-25-1	Green, fatty, grassy, sweet, fresh	HS / SPME / TD	P / NP	(Calkins and Hodgen, 2007; Rochat and Chaintreau, 2005; Ruan et al., 2015; Specht and Baltes, 1994; Van Ba et al., 2010)
Heptanal	111-71-7	Fruity, fatty, sweet, oil, green, rancid	HS / SPME / TD	P / NP	(Calkins and Hodgen, 2007; Rochat and Chaintreau, 2005; Ruan et al., 2015; Specht and Baltes, 1994; Van Ba et al., 2010)

Octanal	124-13-0	Green, lemon, citrus, aldehyde, fatty, orange peel, soapy, honey	HS / SPME / TD	P / NP	(Calkins and Hodgen, 2007; Rochat and Chaintreau, 2005; Ruan et al., 2015; Van Ba et al., 2010)
Nonanal	124-19-6	Sweet, fatty, green, pungent, sea, citrus, citronella grass, floral, grassy, waxy	HS / SPME / TD	P / NP	(Calkins and Hodgen, 2007; Rochat and Chaintreau, 2005; Ruan et al., 2015; Specht and Baltes, 1994; Van Ba et al., 2010)
Decanal	112-31-2	Sweet, fruity, aldehyde, roasty, fatty, rancid, meaty, burnt, overcooked	HS / SPME / TD	P / NP	(Calkins and Hodgen, 2007; Rochat and Chaintreau, 2005; Ruan et al., 2015; Specht and Baltes, 1994; Van Ba et al., 2010)
Undecanal	112-44-7	Sweet, pungent, green, alcoholic, floral, lemon, sea	HS	P / NP	(Rochat and Chaintreau, 2005; Specht and Baltes, 1994)
Furfural	98-01-1	NR	TD	NP	(Ruan et al., 2015)
Benzaldehyde	100-52-7	Almond oil, bitter almond, burning aromatic taste	NR/ SPME / TD	NR / NP	(Calkins and Hodgen, 2007; Ruan et al., 2015; Van Ba et al., 2010)
Phenylacetaldehyde	122-78-1	Sweet, fruity, flowery, honey	HS / SPME	NP	(Gasser and Grosch, 1988; Specht and Baltes, 1994; Van Ba et al., 2010)

Methional	3268-49-3	Cooked potato, meat broth	HS / TD	NP	(Gasser and Grosch, 1988; Ruan et al., 2015; Specht and Baltes, 1994)
2-methylbutanal	96-17-3	Pungent, sweet, roasty	HS / SPME / TD	NP	(Ruan et al., 2015; Specht and Baltes, 1994; Van Ba et al., 2010)
2-nonenal	2463-53-8	Cardboardy, orris, fat, cucumber, paper	NR	NR	(Calkins and Hodgen, 2007)
2-tridecenal	7069-41-2	Sweet, strong, spicy	NR	NR	(Calkins and Hodgen, 2007)
2,4-decadienal	2363-88-4	Deep fat, citrus, orange, grapefruit	NR	NR	(Calkins and Hodgen, 2007)
2,4-nonadienal	6750-03-4	Fat, wax, green, watermelon, geranium, pungent	NR	NR	(Calkins and Hodgen, 2007)
3-methylbutanal	590-86-3	Meaty, fish, rotten, aldehyde, fatty, solvent-like, pungent, green, apple, malt	HS / SPME / TD	P / NP	(Calkins and Hodgen, 2007; Rochat and Chaintreau, 2005; Ruan et al., 2015; Specht and Baltes, 1994; Van Ba et al., 2010)
E-2-hexenal	6728-26-3	Green	NR / SPME	NR / NP	(Van et al., 2012; Van Ba et al., 2010)
E-2-heptenal	18829-55-5	Fatty	NR / SPME	NR / NP	(Van et al., 2012; Van Ba et al., 2010)
E-2-octenal	2548-87-0	Aldehyde, green, floral, dienal, fatty, cardboard, amine, nut	HS / SPME	P / NP	(Calkins and Hodgen, 2007; Gasser and Grosch, 1988;

					Rochat and Chaintreau, 2005; Van Ba et al., 2010)
E-2-nonenal	18829-56-6	Fatty, pungent, fragrant, fruity, roasty, wood, nutty, dienal	HS / SPME	P / NP	(Gasser and Grosch, 1988; Rochat and Chaintreau, 2005; Specht and Baltes, 1994; Van Ba et al., 2010)
E-2-decenal	3913-81-3	Pungent, green, sweet, fruity, fatty, tallow, orange	HS	P / NP	(Calkins and Hodgen, 2007; Rochat and Chaintreau, 2005; Specht and Baltes, 1994)
E-2-undecenal	53448-07-0	Sweet, fruity, fatty	HS	NP	(Specht and Baltes, 1994)
E-2-dodecenal	20407-84-5	Sweet, fruity, roasty, pungent	HS	NP	(Specht and Baltes, 1994)
E,E,2,4-heptadienal	4313-03-5	Aldehyde, green, broth, spicy, nut, fat	HS	P	(Calkins and Hodgen, 2007; Rochat and Chaintreau, 2005)
E,E,2,4-octadienal	30361-28-5	Mild, alcoholic, floral, green, lemon, aldehyde, sea	HS	P	(Rochat and Chaintreau, 2005)
E,E,2,4-nonadienal	5910-87-2	Fatty, dienal, aldehyde, insect, fatty, bad, rancid	HS / SPME	P / NP	(Gasser and Grosch, 1988; Rochat and Chaintreau, 2005; Van Ba et al., 2010)
E,E,2,4-decadienal	25152-84-5	Sweet, rubbery, fatty, pungent, plastic	HS / SPME	P / NP	(Gasser and Grosch, 1988; Rochat and Chaintreau, 2005; Specht and Baltes, 1994; Van Ba et al., 2010)

Ketones [b]

1-octen-3-one	4312-99-6	Fresh, mushrooms, pungent, rubbery, old water, cellar	HS / SPME	P / NP	(Gasser and Grosch, 1988; Rochat and Chaintreau, 2005; Specht and Baltes, 1994; Van Ba et al., 2010)
1-nonen-3-one		Mushroom	HS	P	(Rochat and Chaintreau, 2005)
2-butanone	78-93-3	Chemical, burnt, gas, chocolate	HS	NP	(Machiels et al., 2003)
2-heptanone	110-43-0	Citrus, grapefruit, limonene, floral, cheese, fruity, spicy, cinnamon	HS / SPME	P / NP	(Calkins and Hodgen, 2007; Rochat and Chaintreau, 2005; Van Ba et al., 2010)
2-octanone	111-13-7	Fruity, musty	HS / SPME	NP	(Gasser and Grosch, 1988; Van Ba et al., 2010)
2-nonanone	821-55-6	Hot milk, soap, green, fruity, floral	NR	NR	(Calkins and Hodgen, 2007)
2-decanone	693-54-9	Fruity, musty	HS	NP	(Gasser and Grosch, 1988)
2-undecanone	112-12-9	Fruity	NR	NR	(Van et al., 2012)
2-dodecanone	6175-49-1	Fruity, musty	HS	NP	(Gasser and Grosch, 1988)
2-methyl 3-octanone	923-28-4	Herb, butter, resin, gasoline	NR	NR	(Calkins and Hodgen, 2007)
2,3-butanedione	431-03-8	Sweet, buttery	HS	P / NP	(Rochat and Chaintreau, 2005; Specht and Baltes, 1994)
2,3-pentanedione	600-14-6	Buttery, lemon-like, sweet, fruity	HS	NP	(Specht and Baltes, 1994)
2,5-dimethyl-4-hydroxy-3(2H)-furanone	3658-77-3	Roasted almonds, sweet	HS	NP	(Specht and Baltes, 1994)

3-octanone	106-68-3	Fruity, nutty, moldy, fatty, earthy	HS	NP	(Specht and Baltes, 1994)
3-octen-2-one	1669-44-9	Nut, earthy, spicy, sweet, mushroom	NR	NR	(Van et al., 2012)
3-[(2-furanylmethyl)dithio]-2- butanone	61295-44-1	Onion, burnt rubber, burnt wood	HS	NP	(Mottram, 1998)
3-[(2-furanylmethyl)dithio]-2- pentanone	180031-78-1	Sweet, onion, roast nuts	HS	NP	(Mottram, 1998)
4-methyl-3-penten-2-one	141-79-7	Sweet, chemical	HS	NP	(Machiels et al., 2003)
6-methyl 2-heptanone	928-68-7	Cloves, menthol	NR	NR	(Calkins and Hodgen, 2007)
Dihydro-5-propyl-2(3H)-furanone	105-21-5	Fruity, fatty, sweet, pungent, roasty	HS	NP	(Specht and Baltes, 1994)
Alcohols [c]					
Ethanol	64-17-5	Grilled, acetaldehyde-like			(Van et al., 2012)
Propanol	71-23-8	Alcoholic	NR	NR	(Calkins and Hodgen, 2007)
1-pentanol	71-41-0	Fuel oil, fruit, balsamic	NR / SPME	NR / NP	(Calkins and Hodgen, 2007; Van Ba et al., 2010)
1-hexanol	111-27-3	Woody, cut grass, chemical-winey, fatty, fruity	NR	NR	(Calkins and Hodgen, 2007)
1-heptanol	111-70-6	Fragrant, woody, oily, green, fatty, winey, herb	NR	NR	(Calkins and Hodgen, 2007)
1-octanol	111-87-5	Penetrating aromatic odour, fatty, waxy, citrus, oily, walnut, moss, chemical, metal, burnt	NR / SPME	NR / NP	(Calkins and Hodgen, 2007; Van Ba et al., 2010)

1-octen-3-ol	3391-86-4	Mushroom	NR / SPME	NR / NP	(Calkins and Hodgen, 2007; Van Ba et al., 2010)
2-ethyl-1-hexanol	104-76-7	Resin, flower, green	NR	NR	(Calkins and Hodgen, 2007)
2-hexen-1-ol	928-95-0	Green, sharp, leafy, fruity, unripe banana	NR	NR	(Calkins and Hodgen, 2007)
2-octen-1-ol	22104-78-5	Green, citrus	NR / SPME	NR / NP	(Calkins and Hodgen, 2007; Van Ba et al., 2010)
Pyrazines [d]					
Pyrazine	290-37-9	Nutty, cracker, roasted	SPME	NP	(Van Ba et al., 2010)
2-ethyl-5-methylpyrazine	13360-64-0	Fruity, sweet, pungent	HS / TD	NP	(Ruan et al., 2015; Specht and Baltes, 1994)
2-ethyl-3,5-dimethylpyrazine	13925-07-0	Burnt, fragrant, meaty, green	HS / TD	NP	(Cerny and Grosch, 1992; Ruan et al., 2015; Specht and Baltes, 1994)
2-ethyl-3,6-dimethylpyrazine	13360-65-1	Burnt, roasty, pungent, earthy	HS / SPME	NP	(Specht and Baltes, 1994; Van Ba et al., 2010)
2-ethenyl-5(6)-methylpyrazine	13925-08-1 / 13925-09-2	Roasty break-like, cooked rice, coffee-like, popcorn, smoky	HS	NP	(Specht and Baltes, 1994)
2-ethenyl-3,6(5)-dimethylpyrazine	80935-98-4	Sweet, cooked rice, fatty, pungent	HS	NP	(Specht and Baltes, 1994)

2-isopentyl-3,6-dimethylpyrazine	18433-98-2	Sweet, fragrant, fatty, fruity, pungent	HS	NP	(Specht and Baltes, 1994)
2,3-diethyl-5-methylpyrazine	18138-04-0	Meaty, roasty, fragrant, sweet	HS / TD	NP	(Cerny and Grosch, 1992; Ruan et al., 2015; Specht and Baltes, 1994)
2,5-dimethylpyrazine	123-32-0	Fried rice, popcorn, pungent, green, roasted, earthy, nutty	NR / SPME	NR / NP	(Van et al., 2012; Van Ba et al., 2010)
3-ethyl-pyrazine	13925-00-3	NR	TD	NP	(Ruan et al., 2015)
3-ethyl-2,5-dimethylpyrazine	13360-65-1	NR	TD	NP	(Ruan et al., 2015)
3,5-diethyl-2-methyl-pyrazine	18138-05-1	NR	TD	NP	(Ruan et al., 2015)
Sulphur & nitrogen containing compounds [e]					
Benzothiazole	95-16-9	Stewed, gravy, gas, roasted	HS	NP	(Machiels et al., 2003)
Bis(2-methyl-3-furyl)disulfide	28588-75-2	Meaty, boiled meat	HS	NP	(Gasser and Grosch, 1988; Mottram, 1998)
Bis(2-furanylmethyl) disulfide	4437-20-1	Roast, brazil nuts, baked mushroom	HS	NP	(Mottram, 1998)
Dimethylsulfide	75-18-3	Sweet, gas, fruity	HS	NP	(Machiels et al., 2003)
Dimethyl disulfide	624-92-0	Moldy, pungent, rubbery, onion-like	HS / SPME	NP	(Specht and Baltes, 1994; Van Ba et al., 2010)

Dimethyl trisulphide	3658-80-8	Gas, burnt, gravy, fragrant, musty, roasty, rubbery	HS / SPME	NP	(Machiels et al., 2003; Specht and Baltes, 1994; Van Ba et al., 2010)
Methanethiol	74-93-1	Sweet, gas	HS	NP	(Machiels et al., 2003)
2-acetylthiazole	24295-03-2	Roasted	HS	NP	(Gasser and Grosch, 1988; Machiels et al., 2003)
2-acethylthiophene	88-15-3	Sulphurous, sweet	NR	NR	(Van et al., 2012)
2-formyl-5-methylthiophene	13679-70-4	Sulphurous	NR	NR	(Van et al., 2012)
2-furfurylthiol	98-02-2	Roasty	NR	NR	(Van et al., 2012)
2-mercapto-3-pentanone	17042-24-9	Brothy, mashed potatoes meaty, roast meat	HS	NP	(Mottram, 1998)
2-methyl-3-furanthiol	28588-74-1	Meaty, sweet, sulphurous, roast meat, boiled meat	HS	NP	(Machiels et al., 2003; Mottram, 1998)
2,4-dimethylthiazole	541-58-2	Rubbery, moldy, fruity, pungent	HS	NP	(Specht and Baltes, 1994)
3-mercapto-2-butanone	40789-98-8	Fried onion, sulphury, cooked meat	HS	NP	(Mottram, 1998)
4,5-dimethylthiazole	3581-91-7	Smoky, roasty, fragrant, nutty	HS	NP	(Specht and Baltes, 1994)
Other					
Ethyl acetate	141-78-6	Caramel, sweet	HS	NP	(Machiels et al., 2003)
Butyric acid	107-92-6	Sweet, unpleasant	HS	NP	(Specht and Baltes, 1994)
Hexanoic acid	142-62-1	Goaty	NR	NR	(Calkins and Hodgen, 2007)
Nonanoic acid	112-05-0	NR	TD	NP	(Ruan et al., 2015)

Decanoic acid	334-48-5	NR	TD	NP	(Ruan et al., 2015)
1,3-Bis(1,1-dimethylethyl)benzene	1014-60-4	Cooked beef	NR	NR	(Calkins and Hodgen, 2007)
2-methyl-3-(methyldithio)furan	65505-17-1	Meaty, sulphury, fatty	HS	NP	(Mottram, 1998)
2-[(methyldithio)methyl]furan	57500-00-2	Brothy, spices, roast, fatty	HS	NP	(Mottram, 1998)
2-pentylfuran	3777-69-3	Green bean, butter	NR / SPME	NR / NP	(Calkins and Hodgen, 2007; Van Ba et al., 2010)

Approximate odour threshold [a,e] ppb → ppm; [b] ppm; [d] ppb; HS, headspace; SPME, solid-phase micro extraction; TD, thermal desorption; NR, not reported; P, polar; NP, non-polar.

1.5. Sampling / Extraction methods

The most important step prior to GC determination of an analyte is sample preparation; it is a key step in relation to VOC analysis in meat. Sample preparation involves converting a non-homogenous mixture (meat), into a suitable sample for analysis. Sample preparation procedures for VOC analysis usually involve extraction, concentration, possibly clean-up, and sometimes derivatization to convert non-volatile components to volatile components to transfer to vapour phase prior to injection onto the GC (Smith, 2003). The basic purpose of all extraction methods is to purify (as much as possible) and to concentrate analytes selectively in one or multiple phases. Many different VOC extraction/concentration techniques exist which vary considerably and the choice has a significant impact on the selectivity and sensitivity of an analytical method (Wojnowski et al., 2017) (Table 1.2). It is worth reiterating that no single VOC extraction method can provide a complete representative volatile profile as each has inherent degrees of bias towards specific VOC dependent upon molecular size, polarity and vapour pressure. Other factors concerning potential advantages or disadvantages for each technique relate to the degree of automation/ length of time, cost, sensitivity, practicality, risk assessment, reproducibility, potential for artifact formation and ease of use.

Table 1.2. Comparison of headspace, P&T, HS-SPME and TD GC techniques.

	Matrix	Sensitivity	Range of compounds analysed				Sample weight (g)	Sample procedure	Sample prep. (min)	Sample automate
			Gas	Volatile	Semi-volatile	Non-volatile				
Headspace	L, S	ppm	✓	✓	×	×	0.1-10	E, C, CU, D	5-10	Yes
P&T	G, L, S	ppb	×	✓	✓	×	5-1000	E, C, CU	10-30	No
HS-SPME	G, L, S	ppt	×	✓	✓	×	0.1-10	E, C, CU, D	5-15	Yes
TD	S	ppb	✓	✓	✓	×	0.1-1	E, C, CU, D	1-2	No

P&T, purge-and-trap; HS-SPME, headspace solid-phase micro extraction; TD, thermal desorption; L, liquid; S, solid; G, gas; ppm, parts per million (10^{-6}); ppb, parts per billion (10^{-9}); ppt, parts per trillion (10^{-12}); E, extraction; C, concentration; CU, clean-up; D, derivatization. Adapted from (Manura and Overton, 1999).

For the purpose of this review, some of the most common extraction techniques have been summarised into 3 groups (Fig. 1.2):

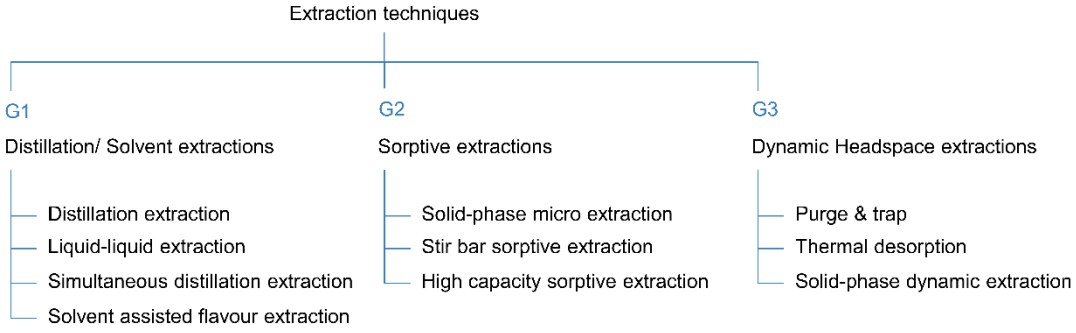


Figure 1.2. Common extraction techniques for VOC in meat.

1.5.1. Distillation/Solvent extraction techniques

Traditionally distillation/ solvent techniques were favoured for volatile extraction in foods, but subsequently as throughput, reproducibility and green

chemistry have become more important this has seen a movement towards more automated and flexible multipurpose extraction/ enrichment techniques.

1.5.1.1. Distillation extraction

Distillation (DE) is a physical separation based on the vaporization of the different components of the mixture to be separated. Typically, a mixture is heated, VOC are enhanced in the headspace, separated, and then condensed back into a liquid. As a result, each component can be recuperated separately in different fractions. The main advantage of all distillation techniques is obtaining a relatively clean liquid extract from the sample. One of the main disadvantages of distillation with a carrier liquid lies in its destructiveness, the need for large volumes of solvents nor does it provide a complete extract as the total amount of a given component is distributed between its liquid and vapour phases (Stauffer et al., 2008). Thus, its more suited for the extraction of semi-volatile and heat stable VOC and less so for more volatile VOC. Distillation is one of the oldest methods used mainly for the extraction of oils from the plants (Hernández-Ochoa et al., 2014).

1.5.1.2. Liquid-liquid extraction

Liquid-liquid extraction (LLE), also known as partitioning, is one of the oldest extraction techniques. The principle of LLE involves transferring an analyte from an aqueous matrix into an extraction solvent, usually water and a nonpolar organic solvent. The extraction solvent used for LLE should be immiscible in the aqueous matrix so that the two liquids can be easily separated. LLE process offers several advantages such as provides high capacity of the extractant with a high selectivity of separation and is relatively simple to perform. Disadvantages of LLE include use of large solvent volumes, long extraction times and multiple extractions may be required in order to remove the majority of the analyte from the matrix (Kyle, 2017). LLE is common in the chemical and pharmaceutical industries and in biotechnology,

but much less so in food processing. Its applications to food are generally restricted to isolated cases, such as edible oils (Berk, 2018).

1.5.1.3. Simultaneous distillation extraction

The simultaneous distillation extraction (SDE) technique combines steam distillation together with solvent extraction. Briefly, the sample is initially prepared in an aqueous solution. An immiscible organic solvent is used as the extractant. Both solutions are heated separately in glass flasks connected to ports of a glass manifold. The sample and extractant vapours are transferred to the central part of the manifold, where they are condensed. The extraction is initiated as the gaseous and liquefied analytes, sample solvent, and extractant come into contact. The sample solvent and the extract undergo phase separation in a U-shaped glass tube. The two liquids are directed to the sample and extractant flasks, respectively (Liao et al., 2020). Despite its general practicality, SDE has several limitations, which hinder its widespread use: it requires practical expertise (a high degree of temperature control, solvent should not mix with the sample matrix), and extraction times are long. However, the SDE technique is particularly suitable for the extraction of VOC present in complex matrices.

1.5.1.4. Solvent assisted flavour evaporation

In the solvent assisted flavour evaporation (SAFE) procedure, odour-active components are distilled under high vacuum. SAFE extraction allows for effective volatile isolation at low temperatures and pressures, thus avoiding potential loss of labile aroma compounds or artifact formation (Wieczorek et al., 2020). However, SAFE is both time consuming and labour intensive. SAFE has been previously considered to be the 'gold standard' for flavour extraction, so it has been employed for the extraction of VOC in a wide range of foods (High et al., 2019; Liu et al., 2018; Wieczorek et al., 2020). It is widely used on GC-O analysis because it can be

undertaken at low temperature to avoid the creation of thermal volatile aroma-active artifacts (Steinhaus, 2020).

As far as the authors can determine little or no published work exists in relation to DE, LLE or SAFE on beef. Studies using LLE in pork (Liu et al., 2019; Zheng et al., 2019) and SAFE in stewed pork (Zhao et al., 2017), heat treated sausages (Ozkara et al., 2019) and dry fermented sausages (Corral et al., 2016a) exist. Only SDE has been used in heated beef (Watkins et al., 2012), as well as in chicken (Zhou et al., 2019) and chicken breast meat (Ayseli et al., 2014).

1.5.2. Sorptive extraction techniques

1.5.2.1. Solid-phase micro extraction

SPME can be employed as a headspace (HS) extraction technique (HS-SPME) or by direct immersion (DI-SPME). In DI-SPME the SPME fiber is inserted into a liquid sample matrix (Kataoka, 2011) in a HS vial under controlled conditions. This approach has limited appeal for VOC analysis in foods due to significant reductions in fibre longevity and the expense of the fibres. However, arguably HS-SPME is the most globally used VOC extraction technique for food research today. The underlying principle of HS-SPME is based on the partitioning of analytes between a coated fibre with appropriate stationary phase(s) and the sample (Merkle et al., 2015). A sample is placed in a sealed HS vial, heated and agitated under controlled conditions until the VOC reach an equilibrium (sometimes referred to HS saturation). At this point a HS-SPME needle is pierced through the vial septum and the fibre is exposed to the gaseous phase in the vial. The next step involves partitioning of the VOC between the sample matrix and the extraction phase(s). The parameters (sample to HS ratio, temperature, time and degree of agitation) of fibre exposure to the HS has a huge impact on the efficiency of the extraction, mainly as the sorptive process is exothermic (Mondello et al., 2005). A main advantage of SPME is the wide range of commercial fibre phases available; apolar polydimethylsiloxane (PDMS) for the extraction of non-polar analytes; more polar carbowax-polyethylene glycol (PEG),

polyacrylate (PA) and dual phases such as PDMS/divinylbenzene (PDMS/DVB) for the extraction of polar analytes (especially phenols and amines, respectively); Carboxen/PDMS (CAR/PDMS) for the extraction of volatile/low molar mass analytes; Carbowax/DVB (CW/DVB) and CW/template resin (CW/TPR) for the extraction of polar analytes (especially alcohols in the first case); and the more generic triple phase fibre DVB/CAR/PDMS for the extraction of a broad range of analytes (Kataoka, 2011). In addition, some phases are available at different thicknesses which can benefit the extraction of some VOC. Once the VOC are extracted, the next step involves desorption of VOC from the fibre in the heated inlet of the GC (Merkle et al., 2015). HS-SPME only requires a small sample size/volume and is suitable for the extraction and concentration of a high number of VOC and semi-VOC (SVOC) (Panighel and Flamini, 2014). A major benefit of HS-SPME is that it can be fully automated and is relatively easy to use and reproduce (Vas and Vékey, 2004a). Volatile and non-polar compounds are extracted faster than SVOC and polar volatiles. Some of the drawbacks of HS-SPME are that stationary phases only cover the scale of polarity of target analytes, or certain molecular weight ranges depending upon the phases used. The fibres can be easily damaged (stripping of coatings, bending of the needle) and are relatively expensive (Nerín et al., 2009). Furthermore, high molecular weight compounds cannot be analysed in combination with GC, and in certain cases, very volatile, polar, or thermally unstable analytes are reported to have low extraction efficiencies (Namieśnik et al., 2000). Another major issue relates to potential analyte displacement. Very volatile compounds are absorbed first and can saturate the fibre due to the relative low capacity of the fibres; also, volatiles with a lower affinity can be displaced over incubation by less volatile compounds with a high affinity for the fibre (Vas and Vékey, 2004b). In an attempt to circumvent this issue, SPME arrow was developed. SPME arrow uses a larger amount of sorbent material to improve detection limits and is less fragile than the traditional SPME fibres (Song et al., 2019). Another development thin-film SPME aims to increase the volume of the extraction phase and therefore the sensitivity of the method by using a thin extraction phase with a large surface area (Bruheim et al., 2003). The VOC responsible for the aroma of pork products, such as fermented sausages (Cano-García et al., 2014; Corral et al., 2013, 2015a, 2015b, 2016a; Di Cagno et al., 2008; Flores et al., 2004; Flores &

Hernandez, 2007; García-Béjar et al., 2020; Kaban & Kaya, 2009; Olivares et al., 2009, 2011, 2015; Stahnke et al., 2002), frankfurters (Chevance and Farmer, 1999), chorizo (Gómez and Lorenzo, 2013), salami (de Campos et al., 2007), ham (Bosse Née Danz et al., 2017; García González et al., 2009; Luna et al., 2006; Martínez-Onandi et al., 2016a, 2016b; Marušić et al., 2014; Sánchez-Peña et al., 2005), pig muscle (Chen et al., 2014)**,** and porcine liver pates (Estévez et al., 2004) have been extensively studied by HS-SPME. HS-SPME has also been successfully applied in the extraction of VOC in raw beef (Acevedo et al., 2012; Mezgebo et al., 2017; Resconi et al., 2018; Sun et al., 2020), roasted and boiled beef (Moon et al., 2006), cooked beef (Ba et al., 2014; Van Ba et al., 2010; Watkins et al., 2012), and minced beef (Argyri et al., 2015). The different HS-SPME parameters used to determine the aroma-relevant VOC in pork and beef products are described in Tables 1.3 and 1.4.

1.5.2.2. Stir bar sorptive extraction

Stir bar sorptive extraction (SBSE) is a solventless sample preparation method for the extraction and enrichment of VOC from aqueous matrices. The method is based on sorptive extraction, whereby the solutes are extracted into a polymer coating on a magnetic stirring rod. The extraction is controlled by the partitioning coefficient of the solutes between the polymer coating and the sample matrix and by the phase ratio between the polymer coating and the sample volume (David and Sandra, 2007). For SBSE, it is known that the analyte recovery in the extraction phase is high. Moreover, this method provides enhanced sensitivity because the extracted fraction can be introduced quantitatively into a GC system by TD. However, extraction of polar/hydrophilic solutes at the trace level is still a serious limitation because this method is generally more selective toward hydrophobic/apolar solutes (Ochiai et al., 2018). Applications of SBSE in food analysis are limited to the analysis of VOC in strawberries (Kreck et al., 2001), grapes (Caven-Quantrill and Buglass, 2006), coffee (Bicchi et al., 2002), beer (Kishimoto et al., 2005), wine (Hayasaka et al., 2003), whiskey (Demyttenaere et al., 2003), sake (Isogai et al., 2005) and vinegar (Guerrero et al., 2007). To date only two studies have been undertaken on beef or

pork; grilled beef (Ruan et al., 2014, 2015) and one on cooked ham (Benet et al., 2015).

Table 1.3. Static headspace conditions used to determine the profile of aroma-relevant volatile compounds in meat products.

	Dried sausages (Salame Milano) (Stahnke et al., 2002)	**Frankfurters** (Chevance and Farmer, 1999)	**Raw ham** (Bosse Née Danz et al., 2017)
Instrument parameters			
Sample quantity (g)	20	50	1
Carrier gas	Nitrogen	Nitrogen	Helium
Valve/vial oven temp (°C)	-	-	40
Sample temp (°C)	42	70	-
Sample equil time (min)	30	30	80
Purge temp (°C)	42	-	-
Purge time (min)	30	-	8
Purge flow (ml/min)	50	50	-
Needle temp (°C)	-	-	75
Trap temp (°C)	-	-	40 low; 280 high
Trap hold time (min)	-	-	5
Trap material	Tenax	-	AirToxic™
Desorb temp (°C)	200	250	-
Desorb time (min)	-	5	0.4
Cold trap (°C)	250	-	-
Transfer line to GC temp (°C)	200	-	120
Pressurize (kPa)	-	-	220
Injector temp (°C)	-	-	150
GC-MS parameters			

Column			
Type	DB-1701 column	CPWax 52CB capillary column CPSil 8CB capillary column	Rtx®-200 fused silica capillary column coated with trifluoropropyl methyl polysiloxane stationary phase
Length (m)	30	50	30
Internal diameter (mm)	0.25	0.32	0.32
Film thickness (μm)	1	-	1
Flow rate	-	-	-
Pressure (kPa)	-	-	70
Oven program	35 °C for 1 min, raised to 175 °C at 4 °C/min, to 250 °C at 10 °C/min held for 5 min	60 °C for 5 min, raised to 220 °C at 4 °C/min	40 °C for 5 min, raised to 80 °C at 10 °C/min held for 3 min; to 180 °C at 10 °C/min held for 3 min; to 240 °C at 15 °C/min held for 5 min
Inlet	-	-	Split flow 15 ml/min
MS			
Transfer line to MS temp (°C)	280	-	150
Source temp (°C)	-	-	240
Ionization potential (eV)	70	-	70
Scan range (m/z; amu)	33-300	-	35-300
Scan time (s)	-	-	0.3
Interscan delay (s)	-	-	0.05

Amu, atomic mass units; *m*/*z*, mass-to-charge ratio.

Table 1.4. Solid-phase microextraction used to determine the profile of aroma-relevant volatile compounds in meat products.

	Fermented sausages (Olivares et al., 2009)	**Fermented sausages** (Flores et al., 2004; Flores and Hernandez, 2007)	**Fermented sausages** (Cano-García et al., 2014)	**Fermented sausages** (Corral et al., 2013; Corral et al., 2015b)	**Fermented sausages** (Di Cagno et al., 2008)	**Fermented sausages** (Corral et al., 2016b)	**(Dry-) Fermented sausages** (Olivares et al., 2011, 2015)	**Dry-fermented sausage Sucuk** (Kaban and Kaya, 2009)	**Dry-fermented sausages** (Corral et al., 2015a)
Instrument parameters									
Sample amount (g)	3	3	7 ml	5	3	5	3	5	5
Septum type	PTFE	PTFE	-	PTFE	PTFE	PTFE	-	PTFE	PTFE
Fibre type	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	PDMS; CAR/PDMS; DVB/CAR/PDMS
Fibre film thickness (µm)	85	85	85	85	-	-	85	75	100/85/50-30
Fibre precondition temp (°C)	-	-	-	-	-	-	-	-	-
Fibre precondition time (min)	-	-	-	-	-	-	-	-	-
Sample equil temp (°C)	30	30	37	37	35	37	37	30	37
Sample equil. Time (min)	60	60	15	30	30	30	60	60	30
Fibre exposure time (min)	180	180	120	180	30	120	180	120	180
GC-MS parameters									
Purge valve mode	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless
Desorb temp (°C)	220	240	220	240/250	220	240	220	250	250
Desorb time (min)	15	6	15	5/15	1	5	15	6	15
Carrier gas	Helium	Helium	Helium	-	Helium	-	-	Helium	Helium
Column									
Type	DB-624 capillary column	DB-624 capillary column	DB-624 capillary column	DB-624 capillary column	Cp-wax 52CB capillary column	DB-624 capillary column	DB-624 capillary column	DB-624 capillary column	DB-624 / HP-INNOWAX column
Length (m)	30	30	3	30	50	-	30	30	30/30

Internal diameter (mm)	0.25	0.25	0.25	0.25	0.32	-	0.25	0.25	0.25/0.25
Film thickness (µm)	1.4	1.4	1.4	1.	1.2	-	1.4	1.4	1.4/0.25
Flow rate (cm/s)	27.3	27.3	34.3	-	-	-	-	-	-
Pressure (psi)	-	-	-	-	-	-	-	-	-
Oven program	38 °C for 13 min, raised to 110 °C at 3 °C/min, to 150 °C at 4 °C/min, to 210 °C at 10 °C/min held for 5 min	38 °C for 13 min, raised to 110 °C at 3 °C/min, to 150 °C at 4 °C/min, to 210 °C at 10 °C/min held for 5 min	38 °C for 13 min, raised to 100 °C at 3 °C/min, to 150 °C at 4 °C/min, to 210 °C at 10 °C/min held for 5 min	38 °C for 13 min, raised to 110 °C at 3 °C/min, to 150 °C at 4 °C/min, to 210 °C at 10 °C/min held for 5 min	40 °C for 2 min, raised to 200 °C at 10 °C/min, to 250 °C at 15 °C/min held for 5 min	-	38 °C for 13 min, raised to 110 °C at 3 °C/min, to 150 °C at 4 °C/min, to 210 °C at 10 °C/min held for 5 min	40 °C for 5 min, raised to 110 °C at 3 °C/min, to 150 °C at 4 °C/min, to 210 °C at 10 °C/min, held for 15 min	38 °C for 13 min, raised to 110 °C at 3 °C/min, to 150 °C at 4 °C/min, to 210 °C at 10 °C/min held for 5 min
Detector type	MSD	MSD	-	FID	FID	-	FID	MSD	FID
MS									
Transfer line temp (°C)	240	240	240	-	-	-	-	24	240
Ion source temp (°C)	-	-	230	-	-	-	-	-	-
Ionization potential (eV)	70	70	70	70	-	-	70	7	70
Scan range (amu)	29-40	29-400	29-400	29-400	-	-	29-400	50-500	29-400
Scan speed	-	-	-	-	-	-	-	-	-

Table 1.4. (cont.)

	Pork fermented sausages (García-Béjar et al., 2020)	**Dry-ripened chorizo** (Gómez and Lorenzo, 2013)	**Salami** (de Campos et al., 2007)	**White dry-cured ham** (Luna et al., 2006)	**Dry-cured ham** (Sánchez-Peña et al., 2005)	**(Serrano) Dry-cured ham** (Martínez-Onandi et al., 2016; MartínezOnandi et al., 2016)	**Iberian ham** (García González et al., 2009)	**Istrian dry-cured ham** (Marušić et al., 2014)	**Porcine liver pate** (Estévez et al., 2004)
Instrument parameters									

Sample amount (g)	50 µl	2	1	3	3	10	3	10 ml	1
Septum type	-	Teflon-rubber	Teflon-rubber	PTFE	PTFE	PTFE	PTFE	PTFE	-
Fibre type	DVB/CAR/PDMS	DVB/CAR/PDMS	CAR/PDMS	DVB/CAR/PDMS	DVB/CAR/PDMS	DVB/CAR/PDMS	DVB/CAR/PDMS	DVB/CAR/PDMS	DVB/CAR/PDMS
Fibre film thickness (µm)	50/30	50/3	75	50/30	50/30	50/30	-	50/30	50/30
Fibre precondition temp (°C)	270	270	300	270	270	-	-	240	220
Fibre precondition time (min)	30	60	60	60	60	-	-	2	45
Sample equil temp (°C)	40	35	35	40	40	35	40	40	60
Sample equil. Time (min)	-	15	15	10	10	60	10	-	30
Fibre exposure time (min)	90	30	30	180	180	60	180	180	-
GC-MS parameters									
Purge valve mode	Splitless; 50 ml/min for 2 min	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless
Desorb temp (°C)	260	260	250	260	260	260	260	230	220
Desorb time (min)	5	5	5	5	5	10	5	2	-
Injection port temp (°C)	-	260	250						220
Carrier gas	Helium	Helium	-	Hydrogen	Hydrogen/Helium	Helium	Hydrogen/Helium	Helium	Helium
Column									
Type	DB-WAX polar column	DB-624 capillary column	CP-Sil 8 CB low bleed/ MS fused silica capillary column	DB-WAX column	DB-WAX column	Zebron 100% polyethylene glycol capillary column	DB-WAX column	DB-5ms capillary column	5% phenyl-95% dimethyl polysiloxane column
Length (m)	60	30	60	60	60	60	60	30	30
Internal diameter (mm)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Film thickness (µm)	0.25	1.	0.25	0.25	0.25	0.50	0.25	0.25	1
Flow rate (ml/min)	1	40 cm/s	-	-	-	1	-	1	1.6
Pressure (psi)	-	-	-	-	15	15	15	-	18.5
Oven program	40 °C for 3 min,	40 °C for 10	40 °C for 2 min,	40 °C for 4 min,	40 °C for 4 min,	45 °C for 16	40 °C for 4 min,	40 °C for 10	40 °C for 10

	raised to 150 °C at 3 °C/min, to 220 °C at 7 °C/min held for 5 min	min, raised to 200 °C at 5 °C/min, to 250 °C at 20 °C/min held for 5 min	raised to 280 °C at 4 °C/min held for 5 min	raised to 91 °C at 1 °C/min, to 201 °C at 10 °C/min held for 10 min	raised to 91 °C at 1 °C/min, to 201 °C at 10 °C/min held for 10 min	min, raised to 110 °C at 4 °C/min held for 9 min, to 230 °C at 15 °C/min held for 3 min, to 250 °C at 10 °C/min held for 2 min	raised to 91 °C at 1 °C/min, to 201 °C at 10 °C/min held for 10 min	min, raised to 200 °C at 5 °C/min, to 250 °C at 20 °C/min held for 5 min	min, raised to 250 °C at 7 °C/min, held for 5 min
Detector type	-	MSD	-	FID	FID	-	FID	-	FID
MS									
Transfer line temp (°C)	-	-	-	-	-	280	-	280	270
Ion source temp (°C)	230	-	-	-	-	230	-	-	-
Ionization potential (eV)	70	70	70	-	-	70	-	70	70
Multiplier voltage (V)	-	1953	-	-	-	-	-	-	1650
Scan range (m/z; amu)	45-550	40-300	-	-	-	33-280	-	50-450	40-300
Scan speed (scan/s)	-	6.34	-	-	-	1.74	-	1	1

Table 1.4. (cont.)

	Pig muscle (Chen et al., 2014b)	**Duroc-Bamei pig** (Chen et al., 2020)	**Cattle beef** (Ba et al., 2014)	**Hanwoo beef** (Van Ba et al., 2010)	**Raw red meat** (Sun et al., 2020)
Instrument parameters					
Sample amount (g)	6	5 ml	1	1	2
Septum type	PTFE	-	PTFE	PTFE	Teflon-silicone
Fibre type	PDMS/Polyacrylate	-	CAR/PDMS	CAR/PDMS	DVB/CAR/PDMS
Fibre film thickness (μm)	100/85		-	75	50/30
Fibre precondition temp (°C)	-	250	250	250	-

Fibre precondition time (min)	-	10	10	10	-
Sample equil temp (°C)	80	55	60	60	50
Sample equil. time (min)	40	15	10	10	-
Fibre exposure time (min)	40	20	30/45/60	60	30
GC-MS parameters					
Purge valve mode	-	-	Split ratio 10:1; split flow 10 ml/min	Split ratio 10:1; split flow 10 ml/min	-
Desorb temp (°C)	250	-	250	250	250
Desorb time (min)	-	-	5	5	5
Injection port temp (°C)	-	240	25	250	-
Carrier gas	Helium	Helium	Helium	Helium	Helium
Column					
Type	PEG capillary column	TG-5MS capillary column	DB-5ms capillary column	DB-5ms capillary column	DB-WAX capillary column
Length (m)	30	30	30	30	30
Internal diameter (mm)	0.25	0.25	0.25	0.25	0.25
Film thickness (µm)	0.25	0.25	0.25	0.25	0.25
Flow rate (ml/min)	0.8	1	1	1	1.2
Pressure (psi)	-	-	7.03	7.03	-
Oven program	35 °C for 5 min, raised to 230 °C at 5 °C/min	50 °C for 2 min, raised to 240 °C at 4 °C/min held for 5 min	40 °C for 5 min, raised to 250 °C at 8 °C/min held for 5 min	40 °C for 5 min, raised to 250 °C at 8 °C/min held for 5 min	50 °C for 4 min, raised to 145 °C at 10 °C/min held for 6 min, raised to 160 °C at 10 °C/min held for 3 min, then 200 °C at 10 °C/min held for 3 min

Detector type	-	-	-	-	MSD
MS					
Transfer line temp (°C)	-	250	250	250	150
Ion source temp (°C)	200	250	-	-	230
Ionization potential (eV)	70	70	70	70	70
Multiplier voltage (V)	-	-	-	-	-
Scan range (m/z; amu)	30-200	40-450	35-300	35-300	30-400
Scan speed (scan/s)	-	0.3	5.27	5.27	-

PTFE, polytetrafluorethylene; DVB/CAR/PDMS, divinylbenzene/Carboxen/Polydimethylsiloxane; FID, flame ionization detector; MSD, mass selective detector; Amu, atomic mass units; *m*/*z*, mass-to-charge ratio.

1.5.2.3. High capacity sorptive extraction

High capacity sorptive extraction (Hi-Sorb) is relatively new and innovative, semi-automative approach for the analysis of VOC by TD GC-MS. Hi-Sorb probes are able to sorptively extract VOC and SVOC from a range of sample types, both from the HS or by direct immersion (DI) of liquids, similar to SPME or SBSE. This is an easy-to-use, high capacity highly sensitive approach without the expense of time associated with solvents extraction methods. Unattended sample preparation using the Hi-Sorb Agitator, or with a semi- or fully automated system such as Centri (Markes International, Bridgend, UK) in tandem with a TD, ensures maximum productivity, while reusable probes and tubes minimize the cost per sample. It is highly reproducible and takes high sample capacity. Hi-Sorb in combination with TD enables venting of unwanted analytes, VOC enrichment and recollection. Depending upon the degree of automation, it can be relative expensive but the probes are reusable. Initially it was only available with PDMS but is now also available with PDMS/CWR, PDMS/DVB and DVB/CWR/PDMS. Typical Hi-Sorb applications include aromatics in coffee, lactones in milk, bacterial VOC in bodily fluids, esters in whisky (Markes International, 2021b), and whole milk powder (Cheng et al., 2021), but no research has been carried out on meat to date.

1.5.3. Dynamic headspace extraction techniques

DHS is one of the most widely used methods for volatile fraction analysis (Wojnowski et al., 2017). DHS transfers VOC from a solid or liquid matrix to the vapour phase by heating in a controlled inert gas flow. The carrier gas is either swept over the sample surface or bubbled through the sample (Rouseff and Cadwallader, 2001). The concentration and volatility of the analytes, sample size, sorbent type, extraction time, and carrier gas flow determine the effectives of the extraction (Wojnowski et al., 2017). When a sample presents analytes at ultra-trace levels in the parts-per-billion (ppb) to parts-per-trillion (ppt) range, or where an exhaustive extraction of the analyte is required (Lubes and Goodarzi, 2017), DHS is particularly

useful (Wojnowski et al., 2017) as the capacity of the sorbent phase in relation to the sample mass/volume is high. DHS offers many advantages: easy sample preparation, complete automation, solvent-less, eliminates contamination, and a wide range of sorbent phases are available (Ochiai et al., 2014). It is also possible to vent unwanted analytes, enrich extracts and recollect extracts. Overall, the potential to optimise this system to target specific analytes is large (Narváez Rivas et al., 2012). However, the main issue with DHS is moisture control. DHS systems can manage moisture control *via* dry purging, but in samples with significant levels of moisture it is more problematic, as moisture if introduced to the GC column can adversely impact on its lifespan, and increase background noise through phase deterioration and damage the MS-detector (Narváez Rivas et al., 2012). DHS has been used to determine distinct aroma flavours of pork sausages (Hagen et al., 1996; Nam et al., 2011; Wagner et al., 2008), frankfurters (Chevance and Farmer, 1999), ham (Liu et al., 2014; Timón et al., 2001) and *lacon* (Lorenzo et al., 2013), as well as cooked beef (Nakai et al., 1999; Piao et al., 2019). The conditions under which these foods were analysed are listed in Table 1.5.

Table 1.5. Dynamic headspace conditions used to determine the profile of aroma-relevant volatile compounds in meat products.

	Dry fermented sausages (Hagen et al., 1996)	**Irradiated sausages** (Nam et al., 2011)	**Frankfurters** (Chevance and Farmer, 1999)	**Seasoned sausages (salami)** (Wagner et al., 2008)	**Dry-cured Iberian ham** (Timón et al., 2001)	**Jinhua ham** (Liu et al., 2014)	**Dry-cured lacon** (Lorenzo et al., 2013)	**Hamburger patty** (Nakai et al., 1999)	**Korean cattle, Holsteins, Angus steers** (Piao et al., 2019)
Instrument parameters									
Sample amount (g)	10	1	50	-	8	-	10	1	10
Carrier gas	Helium	Helium	Nitrogen	-	Helium	Nitrogen	Helium	-	-
Sample temp (°C)	-	-	70	-	40	50	60	-	85
Preheat time (min)	-	-	30	-	10	30	5	5	30
Purge temp (°C)	-	40	-	40	35	-	-	52-53	50 °C for 3 min, raised to 240 °C at 5 °C/min held for 9 min
Purge time (min)	45	15	-	206	30	20	20	15	-
Purge flow (ml/min)	40	40	50	-	-	100	60	-	-
Trap temp (°C)	-	-	-	-	-	-	-	20	-
Trap time (min)	-	-	-	-	-	-	-	-	-
Trap material	Tenax	Tenax/silica/ charcoal	Tenax	-	Tenax	Tenax	Tenax	-	-
Desorb temp (°C)	220	220	-	-	225	280	225	185	-
Desorb time (min)	-	1	-	-	2	-	-	-	-
Desorb flow rate (ml/min)	-	-	-	-	-	-	300	-	-
Transfer line to GC temp (°C)	-	-	-	-	200	-	-	-	-
Injector temp (°C)	-	-	-	250	-	250	220	-	-
Detector temp (°C)	-	-	-	250	280	-	260	250	-
GC-MS parameters									
Carrier gas	Helium	Helium	-	Helium	-	Helium	Helium	-	Helium

Column									
Type	DB-5 apolar capillary column	HP-Wax; HP-5	CPWax 52CB; CPSil 8CB capillary column	DB-Wax fused capillary column	DB-5 fused Silica capillary column	DB-5ms UI DB-WAX	DB-624 capillary column	DB-624 megabore capillary column	HP-PLOT Q
Length (m)	60	7.5; 30	50	30	50	30	30	75	30
Internal diameter (mm)	0.33	0.25; 0.25	0.32	0.25	0.32	0.25	0.25	0.53	0.53
Film thickness (µm)	-	0.25; 0.25	-	0.5	1.05	0.25	1.4	3	0.25
Flow rate (ml/min)	1.2	-	-	1	-	1.2	36 cm/s	-	20
Oven program	40 °C, raised to155 °C at 3 °C/min	7 °C for 2.5min, raised to 25 °C at 3 °C/min, to 120 °C at 10 °C/min, and to 200 °C at 20 °C/min	60 °C for 5 min, raised to 220 °C at 4 °C/min	35 °C for 5 min; raised to 80 °C at 2 °C/min; to 200 °C at 4 °C/min	35 °C for 10 min; raised to 150 °C at 7 °C/min; to 250 °C at 20 °C/min held for 5 min	40 °C for 3 min, raised to 200 °C at 5 °C/min, to 230 °C at 5 °C/min held for 3 min	40 °C for 2 min, raised to 100 °C at 3 °C/min, to 180 °C at 5 °C/min, to 250 °C at 9 °C/min held for 5 min	40 °C for 30 min, raised to 80 °C at 10 °C/min, to 220 °C at 4 °C/min held for 20 min	35 °C for 5 min, raised to 180 °C at 7 °C/min, raised to 250 °C at 5 °C/min held for 21 min
Inlet	Injection split 1 ml/min	-	-	Splitless	-	Splitless; Inlet heating rate 12 °C/min to 260 °C	Split ratio 1:20	-	210 °C
MS									
Transfer line to MS temp (°C)	-	155	-	-	280	280	-	-	250
Ionization potential (eV)	70	70	70	-	-	70	70	-	-
Scan range (m/z; amu)	-	46.1-550	35-250	-	-	45-650	40-300	-	30-250
Scan speed (scan/s)	-	2.94	-	-	-	-	6.34	-	0.2
Quadrupole temp (°C)	-	-	-	-	-	-	-	-	-
Ion source temp (°C)	-	-	-	-	-	230	-	-	250

Amu, atomic mass units; *m*/*z*, mass-to-charge ratio.

1.5.3.1. Purge and trap

P&T was the method of choice for extracting and concentrating VOC from almost any matrix (Sigma-Aldrich, 1997) prior to the advent of more easier to use automatable techniques, especially sorptive extraction techniques. P&T is a DHS sampling technique where the inert gas is passed through the sample onto a trap containing adsorbent or absorbent materials which retain the VOC (Narváez Rivas et al., 2012). The trap is rapidly heated to desorb the VOC which are then transferred to the GC column (Sigma-Aldrich, 1997). The main advantages of P&T are enhanced sensitivity for highly volatile compounds, the absence of extended heating times, and reproducibility (Soria et al., 2008). The disadvantages are mainly related to moisture management, control of foaming, the semi automotive approach, and VOC carry over. P&T has been used for the analysis of raw beef (Insausti et al., 2002a), cooked beef (Gorraiz et al., 2002; Insausti et al., 2005; Machiels et al., 2003) and grilled beef (Ruan et al., 2015), but also for pork sausages (Hierro et al., 2005; Partidário et al., 2006; Procida et al., 1999) and ham (Carrapiso et al., 2002; Fulladosa et al., 2010). The extraction/concentration conditions of the P&T technique used to determine the volatile profile of the meat products are shown in Table 1.6.

Table 1.6. Purge and trap conditions used to determine the profile of aroma-relevant volatile compounds in meat products.

	Dry-fermented sausages (Hierro et al., 2005)	**Dry-fermented sausages** (Partidário et al., 2006)	**Seasoned sausages (salami)** (Procida et al., 1999)	**Dry-cured ham** (Fulladosa et al., 2010)	**Iberian ham** (Carrapiso et al., 2002)	**Raw beef** (Insausti et al., 2002a)	**Cooked beef (bull and heifer)** (Gorraiz et al., 2002)	**Cooked beef (cattle)** (Insausti et al., 2005)
Instrument parameters								
Sample amount (g)	25	1	10	1	6	15	10	10
Carrier gas	Nitrogen	Nitrogen	Helium	Nitrogen	Helium	Helium	Helium	Helium
Sample temp (°C)	30	45	70	35	50	30	70	70
Preheat time (min)	30	5	15	30	5	-	-	-
Purge temp (°C)	30	-	-	-	-20	30	-	-
Purge time (min)	60	10	-	-	30	16	10	10
Purge flow (ml/min)	40	43	-	-	40	40	40	40
Trap temp (°C)	-	-	240	-	220	-	30	30
Trap time (min)	-	-	-	-	2	-	-	-
Trap material	Tenax	Tenax/silica gel/charcoal	-	-	Tenax/silica gel/charcoal	Tenax	Tenax	Tenax
Desorb temp (°C)	-	225	-	150	220	180	180	180
Desorb time (min)	5	2	-	20 s	-	4	4	4
Desorb flow rate (ml/min)	-	-	-	-	-	40	40	40
Transfer line to GC temp (°C)	-	-	-	-	210	-	-	-
Injector temp (°C)	-	210	-	-	230	250	150	150
Detector temp (°C)	-	220	-	-	250	-	250	-
GC-MS parameters								
Carrier gas	-	Helium	-	Helium	Helium	Helium	Helium	Helium

Column								
Type	CP-Sil 8 CB low bleed/ MS fused silica capillary column	DB 23 column	PS 264 capillary fused-silica column	DB-624 column	HP-5 fused capillary column; HP-FFAP fused capillary column	HP-5 capillary column	HP-5 capillary column	HP-5 capillary column
Length (m)	60	30	50	30	50/30	50	50	50
Internal diameter (mm)	0.25	0.32	0.32	0.25	0.32	0.32	0.32	0.32
Film thickness (µm)	0.25	0.25	3	1.4	1.05/0.25	1.05	1.05	1.05
Flow rate (ml/min)	-	-	-	1	-	1	1	1
Pressure (psi)	-	70 kPa	-	-	-	6	10	10
Oven program	40 °C for 2 min, raised to 280 °C at 4 °C/min held for 5 min	35 °C for 8 min, raised to 220 °C at 10 °C/min	40 °C for 6 min, raised to 180 °C at 5 °C/min held for 5 min, to 200 °C at 7 °C/min held 2 min, to 240 °C at 7 °C/min held 5 min	50 °C for 5 min, raised to 225 °C at 5 °C/min held for 10 min	35 °C for 5 min, raised to 150 °C at 10 °C/min, to 250 °C at 20 °C/min held for 10 min	35 °C for 15 min, raised to 220 °C at 8 °C/min held for 15 min	35 °C for 15 min, raised to 220 °C at 8 °C/min held for 5 min	35 °C for 15 min, raised to 220 °C at 8 °C/min held for 5 min
Inlet	-	-	-	Split ratio 1:25 at 150 °C	-	Split ratio 5:1	Split ratio 1:5	Split ratio 1:5
MS								
Transfer line to MS temp (°C)	-	-	250	225	280	-	-	-
Ionization potential (eV)	70	-	70	70	70	70	70	70
Multiplier voltage (V)	-	-	-	-	1675	2000	3000	2000
Scan range (m/z; amu)	-	-	29-300	35-350	30-300	30-250	30-250	30-250
Scan speed (scan/s)	-	-	0.5	1	1	3.32	3.32	3.32
Quadrupole temp (°C)	-	-	-	-	-	108	108	108
Ion source temp (°C)	-	-	-	-	-	230	230	230

Amu, atomic mass units; *m*/*z*, mass-to-charge ratio.

1.5.3.2. Thermal desorption

Thermal desorption (TD) is a common DHS technique, frequently used for VOC and SVOC analysis. In its simplest form, TD involves heating a sample and collecting VOC in a tube packed with absorbent and adsorbent phases in a gas stream. Like P&T the tube is desorbed directly to the GC. In summary, volatiles are desorbed from the sample and introduced in the GC by an online process, thus saving time, sample handling, and reagents. In many TD applications a focussing trap is present inline prior to transfer to the GC, to reduce peak broadening and further enrich the volatiles as multiple tubes can be collected into the same focussing trap. Focusing traps are often made of the same phases as TD tubes and therefore different options are also available to discriminate or concentrate as desired. TD also has a very low risk of sample contamination because a minimal manipulation of the sample is required (Valero et al., 2001). Many TD systems enable re-collection, running blanks, automated leak detection with a very high degree of control over gas flow, backflushing, pressure and temperature control. This technique has been used for qualitative analysis of VOC and SVOC, as a wide range of phases are available. Recoveries of both thermally labile and low volatility compounds have been found to be effective using TD (Özel et al., 2006). TD has not been used extensively in meat products (Markes International, 2019), possibly due to moisture control issues, however some studies exist on beef (Spanier et al., 1994), grilled beef (Ruan et al., 2015) and dried sausages (Stahnke et al., 2002). The extraction/concentration conditions of the TD technique used to determine the volatile profile meat products are shown in Table 1.7.

Table 1.7. Direct thermal desorption conditions used to determine the profile of aroma-relevant volatile compounds in meat products.

	Dried sausages (Salame Milano) (Stahnke et al., 2002)	**Grilled beef** (Ruan et al., 2015)
Instrument parameters		
Sample quantity (g)	20	Stir bars
Carrier gas	Nitrogen	Helium (1.2 ml/min)
Sample temp (°C)	42	
Sample equil time (min)	30	
Purge temp (°C)	42	
Purge time (min)	30	
Purge flow (ml/min)	50	
Trap material	Tenax	
Desorb temp (°C)	200	40 °C for 1min, 200 °C for 5 min
Cold trap (°C)	250	
Transfer line to GC temp (°C)	200	
GC-MS parameters		
Column		
Type	DB-1701 column	HP-5 MS fused-silica capillary column
Length (m)	30	30
Internal diameter (mm)	0.25	0.25
Film thickness (µm)	1	0.25
Oven program	35 °C for 1 min, raised to 175 °C at 4 °C/min, to 250 °C at 10 °C/min held for 5 min	50 °C for 1min, raised to 100 °C at 10 °C/min held for 2 min, to 280 °C at 30 °C/min held for 1 min
MS		
Transfer line to MS temp (°C)	280	
Ionization potential (eV)	70	70

1.5.3.3. Solid-phase dynamic extraction

Solid-phase dynamic extraction (SPDE) is based on the same principles as SPME, but uses an internally coated steel needle instead of a fibre for the extraction and pre-concentration of VOC from the sample (Castro et al., 2015). SPDE can be used in HS or DI modes. The syringe plunger is moved up and down several times for a dynamic extraction of the sample, and the analytes are sorbed in the internal coating. After a predetermined number of extraction cycles (aspirating and dispensing), the analytes are thermally desorbed from the coating in the GC injector (Jochmann et al., 2006). SPDE offers several inherent advantages over SPME and SBSE, mainly that SPDE needle coatings possess around four to six times larger extraction phase volumes (4.5 µ l of the polymer) compared with a 100-µm SPME fibre (0.6 µ l of PDMS) (Christ et al., 2006). SPDE has been applied to a wide range of target compounds and different food matrices (Bicchi et al., 2004). One study (García-Lomillo et al., 2016) utilised SPDE to quantify pyrazines in barbecued beef patties using PDMS and activated carbon, but otherwise very little studies appear to have used this extraction technique for the analysis of volatile compounds in beef or pork. This may be due to difficulties in preventing moisture ingress into the needle that may adversely impact VOC interaction with the phase, and/or optimising the extraction methods in relation to number of pump strokes and volume, extraction temperatures, times and desorption.

1.6. Gas chromatography

GC is a common type of chromatography used to separate and analyse VOC, SVOC or even some non-volatile compounds by derivatization (Janssen et al., 2020). Nowadays, GC continues to play an important role in the analysis of numerous food components (Zhong and Wang, 2019). It is always necessary to include a prior step involving the extraction and pre-concentration of the volatile fraction (Valero et al., 2001). Once the VOC have been isolated from their matrix, their analysis is carried out by GC with an appropriate detector. A variety of columns with different

properties are available. The use of fused-silica capillary columns with improved surface inertness, thermal stability and resolution best fulfils most of the analysis requirements (good resolution, high repeatability and reproducibility, high precision, minimum decomposition) (Al-Bukhaiti et al., 2017). The selection of the proper capillary column for any application should be based on four significant factors which are stationary phase (ranging from nonpolar to polar or highly polar), column internal diameter, film thickness, and column length. Each stationary phase provides a specific combination of interactions for each chemical class of analytes. A non-polar column is best for the analyses of non-polar compounds, generally composed only of carbon and hydrogen atoms and contain carbon-carbon single bonds. Polar columns most effectively separate polar compounds, composed primarily of carbon and hydrogen atoms, but also contain one or more atoms of bromine, chlorine, fluorine, nitrogen, oxygen, phosphorus, or sulphur (Sigma-Aldrich, 2013). Hydrogen, helium or nitrogen can be used as carrier gas, however traditionally helium is favoured as it provides a faster flow than nitrogen and the flammability of hydrogen restricted its use (Narváez Rivas et al., 2012). However, as helium gets more expensive as supplies dwindle, hydrogen usage continues to rise, as it is relatively inexpensive when generated in-house and built-in safety devices in hydrogen generators and in GCs significantly reduce the hazard.

1.7. Identification of volatiles – detection systems

GC can be used with a wide range of ionization detectors, the most common are flame ionization detectors (FID), electron capture detectors (ECD) or thermal ionization detectors (TID). However, for VOC analysis the preferred choice, by far, is coupling GC with MS detectors. Although relatively expensive compared to ionization detectors, it is imminently suited as gas flows are complementary, the systems are easily controlled/managed, sample extracts for volatile analysis are relatively clean, and information on compound identity using mass to charge ratio and retention times are easily achieved (Janssen et al., 2020). Different mass ionization processes exist, but electron ionization at 70 V is the most common. The main advantage of 70

V mass ionization energy is the wide range of commercial mass libraries of volatile compounds that are available, which aids identification of VOC. The types of mass analysers widely available for GC-MS today, include single-quadrupole (Q) MS, time-of-flight (TOF) MS, triple-quadrupole (TQ) MS, and high-resolution systems such as, QTOF MS and Orbitrap MS. High-resolution MS systems (QTOF and Orbitrap) are becoming more popular due to their higher scanning speeds and increased sensitivity and dynamic range. However, due to costs, most volatile analysis of foods is still undertaken using Q MS. The main disadvantages of GC-MS in general remain the costs, but also the time required and the need for experienced highly trained operatives to maximise the potential of this hyphenated technique (Janssen et al., 2020; Lubes and Goodarzi, 2017).

The compounds can be identified (a) by comparison with commercial reference standards (Narváez Rivas et al., 2010b), (b) by comparison of Kovats indices with those described by other authors (Andrade et al., 2009), and (c) by comparison of their mass spectra with those contained in libraries, such as NBS (National Bureau of Standards) (López et al., 1992), Wiley (Narváez Rivas et al., 2010b), NIST/EPA/NIH (Narváez-Rivas et al., 2010a), FFNSC 3 (Shimadzu, n.d.), MassLab v.1.3 (Sánchez-Peña et al., 2005), Aroma Office ^{2}D (Gerstel, n.d.), mzCloud mass spectral library (ThermoFisher Scientific, n.d.) or in-house libraries constructed using target compounds.

1.8. Gas chromatography olfactometry

The extraction techniques as mentioned above in combination with GC-MS can help researchers to identify the VOC in meat samples, but they cannot identify the aroma characteristics of the VOC involved, nor identify those impacting sensory perception, or their perceived intensity. This can only be achieved via GC-O. GC-O utilizes the human nose as an additional detector in combination with mass spectrometry or flame ionization detection (Welke et al., 2021).

Experienced trained assessors describe each perceived odour, and can provide additional information on its relative intensity (Carrapiso et al., 2010). There are

three main GC-O methods used in the characterization of odour-active compounds, which are based on the principle of detection frequency, time-intensity, and dilution to threshold (Welke et al., 2021). Detection frequency provides the number or percentage of individuals who sensed the odour of a compound (Welke et al., 2021). Time-intensity methods provide data on the description and intensity of the odour evaluated using a quantitative scale (Welke et al., 2021). Dilution to threshold consists of preparing a series of sample dilutions and each dilution is analysed until no odorant could be detected (Welke et al., 2021). The main disadvantages are that only aromas from individual or co-eluting VOC can be determined and not the global aroma profile of the sample, the time taken to train panellists, and having to evaluate samples in triplicate with multiple assessors. Co-elutions can hide the true aroma of each single compound, influencing perception. Hence, more novel analytical approaches such as GCxGC may be used (Nicolli et al., 2020), but it can be difficult to get sufficient VOC concentration in the second dimension for olfactometry analysis. Synergies with other compounds from the matrix and/or the change of their sensory characteristics by their concentration may also influence the analysis making only a small percentage of volatiles odour-active (Narváez Rivas et al., 2012). Often with GC-O characteristic aromas are detected without any corresponding peak, which highlights the sensitivity of the human nose over the detector. However, combining GC-O with descriptive sensory analysis, in short, applying sensometrics can provide more accurate information in relation to the overall aroma of the food (Welke et al., 2021). The volatile profile of cooked meat was assessed by GC-O (Khan et al., 2015), but it appears that significant scope exists to further utilise this approach to determine factors that influence the sensory characteristic of beef aroma. The choice of VOC extraction method is important in GC-O, as normally you are trying to avoid the creation of odour-active thermal artifacts or to lose thermally labile VOC (Steinhaus, 2020). This is why extraction techniques that use solvents rather than temperature tend to be favoured for GC-O. However, in meat applications temperature is needed as you are often trying to elucidate the key flavour compounds in cooked product, hence trying to mimic the cooking parameters used to prepare the product for sensory evaluation.

1.9. Future trends

The future trends in GC or GC-MS should continue to focus on flexibility of VOC extraction/concentration, platform miniaturization, increased sensitivity, automation and better resolution. There is still work to be done around capabilities for increasingly lower detection limits, through more efficient generation and transfer of ions, and in the chromatography process itself. Firstly, high sensitivity is required to ensure that trace compounds are detected because even a trace amount of a compound with a low odour threshold can have a large impact on the overall aroma. Secondly, the diverse range of chemical classes requires advanced separations to resolve co-elutions and provide confident identification of the analytes present. Comprehensive GCxGC with time-of-flight mass spectrometry (TOF MS) or Orbitrap MS can tackle this challenge by coupling two columns of different selectivity to separate the analytes based on two different chemical properties (e.g., volatility and polarity). These detectors feature both high resolution as well as high mass accuracy and can be used to distinguish between VOC with the same nominal mass, determine elemental compositions and identify unknowns. The full automatization of sample preparation, VOC extraction and concentration using multiple phases in tandem with multiple detection channels would help to address the need for more specific and confident results with shorter turnaround times. Finally, more sophisticated data analysis software is required to quickly identify subtle differences between aroma profiles and find associations between aroma compounds and sensory perception.

With respect to beef and pork flavour, consumers are becoming more discerning about quality and production history in addition to price. More research in customising the beef and pork flavour through production processes and genetics is useful (Judge et al., 2021; Lebret et al., 2011; O'Sullivan et al., 2021). Studies could focus on altering the fatty acid composition of the meat by specialised formula feed with the purpose of manipulating the flavour of the meat. Moreover, the actual trend to demand natural flavour ingredients in foods makes flavour scientist to look for precursors and biotechnology processes for the production of natural meat flavours.

1.10. Concluding remarks

Beef and pork are reservoirs of VOC that can contribute to the development of complex flavours during cooking. All the volatile components that are responsible for the special flavour of beef and pork appear to fall into the following chemical classes: aldehydes, ketones and alcohols, with some acids, esters, furans and N-/S-containing compounds too (Chen et al., 2014, 2019). Among them, the impact of sulphur-containing compounds is of main importance in beef and pork aroma, but the contribution of other aroma VOC should not be underestimated due to possible synergies amongst other VOC. The key volatiles of cooked beef include: octanal, nonanal, (E,E)-2,4-decadienal, methanethiol, methional, 2-furfurylthiol, 2-metyl-3-furanthiol, 3-mercapto-2-pentanone, and 4-hydroxy-2,5-dimethyl-3-(2H)-furanone (Van et al., 2012). These compounds occur also in cooked pork differing in concentrations. Other compounds thought to contribute to the flavour of pork are hexanal, 1-nonanal, 3-methyl-1-butanol, 1-penten-3-one, 2-pentyl-furan, N-morpholinomethyl-isopropyl-sulphide and methyl butyrate (Chen et al., 2019). Thousands of VOC have been identified in beef and pork, but it is the use of olfactometry methodologies and MS that contribute to increase the knowledge of the importance of these in relation to consumer preference.

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Chapter 2

Chapter 2. Literature review: Volatile compounds of six species of edible seaweed

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2.1. Abstract

Seaweeds are widely distributed throughout the world. Many seaweeds are of commercial importance with some consumed directly or used as an ingredient as they have functional, nutritional and/or organoleptic properties. Consumer acceptance of food is closely related to its sensory properties, of which flavour is of prime importance. A significant contributor to flavour is the aromatic volatile components present. This review focusses on the volatile components identified in commercially important edible macroalgae species consisting of four brown (*Himanthalia elongata*, *Laminaria* spp. including *L. ochroleuca*, and *Undaria pinnatifida*) and two red species (*Porphyra umbilicalis* and *Palmaria palmata*). In excess of 200 volatile compounds have been identified consisting of hydrocarbons, alcohols, aldehydes, ketones, acids, esters, furans, phenols and sulphur- containing compounds, among others present in minor quantities. The extraction/ concentration conditions, chromatography and detection methodologies applied varied and impacted on the volatiles identified due to differences in their hydrophobicity, molecular weight and vapour pressure. This review highlights that considerably more information is required to identify volatile aromatic compounds in edible macroalgae and to identify those most likely to impact sensory perception. Such information could be used to aid new product development or widen applications of these seaweeds in the food or beverage sectors.

2.2. Introduction

Seaweed or macroalgae are a large diverse group of macroscopic, eukaryotic, photosynthetic, marine organisms. Ten thousand different species have been described to date (Garcia-Vaquero et al., 2017). According to their pigmentation, seaweeds are classified into three higher taxa, which include brown seaweed (Phaeophyceae), green seaweed (Chlorophyceae), and red seaweed (Rhodophyceae) (Yu et al., 2014). Seaweeds compose a credible source of functional ingredients with bioactive and volatile aromatic compounds (Samarakoon and Jeon, 2012). There are currently 15-20 species of edible seaweed commercially available for consumption in Europe (Peinado, Girón, et al., 2014). Seaweeds are widely consumed, especially in Asian countries, fresh, dried, or as ingredients in prepared foods (Kılınç et al., 2013). They represent a food group that is not normally ingested in unprocessed form in Western societies, where algae remain of minor importance in spite of proven nutritional benefits (MacArtain et al., 2007).

Researchers worldwide have placed great importance on the chemical composition and bioactive components in seaweeds (Kumar et al., 2008; Marsham et al., 2007). Their use as functional ingredients has instigated new developments in food processing (Bouga and Combet, 2015; Roohinejad et al., 2017), one of which is in meat product formulation (Kılınç et al., 2013). Seaweeds have significant potential for use in processed foods as they can provide or not strong odours and characteristic marine flavours (Ferraces Casais et al., 2013). Researchers have also investigated the volatile fraction of some edible seaweed species, generally *via* gas chromatography mass spectrometry (GC-MS). Volatile compounds from macroalgae can be extracted utilizing several processes, the aim of which is to obtain an aromatic profile of the species in fresh or processed form (Gressler et al., 2009). The extraction and concentration of volatile compounds from seaweeds has been conducted using headspace solid-phase microextraction (HS-SPME) (Balbas et al., 2015; López-Pérez et al., 2017), dynamic headspace (DHS) extraction (Ferraces Casais et al., 2013) and static headspace (SHS) extraction (de Alencar et al., 2017), simultaneous distillation extraction (SDE) (Kamenarska et al., 2006a), supercritical fluid extraction (SFE)

(Michalak et al., 2015), pressurized solvent extraction (PSE) (Merichel Plaza et al., 2010), multiple headspace sorptive extraction (MHSSE) (Maruti et al., 2018) and pre-evaporation (Beauchêne et al., 2000). It is well established that even though multiple extraction techniques are available for the analysis of volatile organic compounds none as yet can provide a complete volatile profile as each has inherent bias for certain chemical classes based on volatility, polarity, vapour pressure and molecular weight (Gressler et al., 2009).

Consumers' acceptance of food is intimately related to its flavour. Therefore, volatile components are very important parameters in food quality and flavour (Ferraces Casais et al., 2013). Volatile organic compounds are molecules with high vapour pressure, moderate hydrophilicity and low molecular weight. Hydrocarbons, ketones, aldehydes, alcohols, carboxylic acids, esters, halogenated compounds, sulphur compounds, furans, pyrazines, pyridines, amines and other volatile compounds comprise a broad range of volatile metabolites present in seaweeds (Gressler et al., 2011). There are significant differences in volatile compounds between seaweed species. The species, its geographical origin and processing parameters are known to influence its volatile profile (López-Pérez et al., 2017).

The main aim of this review is to provide insights into the volatile analyses of six commercially important brown and red seaweed species used in the food industry. The most widely harvested, due to their abundance and accessibility, are the brown *Laminaria* spp. and the red *Palmaria palmata* seaweeds. This review focuses on the volatile fraction of edible seaweeds belonging to four species (*Himanthalia elongata*, *Laminaria* spp. including *Laminaria ochroleuca*, and *Undaria pinnatifida*) of brown seaweed, and two species (*Porphyra umbilicalis* and *Palmaria palmata*) of red seaweed due to their abundance in Irish waters (Irish Seaweeds, n.d.; Rajauria et al., 2017). A data search using the major databases (PubMed, ScienceDirect, Springerlink, ResearchGate) with the keywords ("macroalgae", "seaweeds", "volatile compounds", "brown kelp", "*Himanthalia elongata*", "*Laminaria japonica*", "*Laminaria ochroleuca*", "*Palmaria palmata*", "*Porphyra umbilicalis*", "*Undaria pinnatifida*", "Winged kelp") highlighted that there is a lack of information on the volatile compounds for these seaweed species and therefore this review is timely as it provides a relevant summary of up to date information.

2.3. Sensory properties of seaweed volatile compounds

Volatile compounds perceived simultaneously define the odour of a food product (Audouin et al., 2001). Over 10,000 volatile compounds have been detected in foods, but only a few hundred (5-10 %) have any significance as odour compounds (Jelen and Gracka, 2016). Key odour compounds have odour threshold values in a very broad range (mg/L - pg/L) and two key odour features, odour threshold and compound concentration, must be taken into account when discussing the odour impact of compounds in foods (Jelen and Gracka, 2016). The odour activity value (OAV) estimates the importance of a flavour compound in a food, based on the ratio of its concentration to its odour threshold concentration in that food. Only compounds which exceed the threshold level in a food will impact on flavour and thus sensory perception (Audouin et al., 2001). Conceptually, the larger the OAV, the more likely that compound will contribute to the overall odour of a complex odour mixture (Yang et al., 2015).Compounds with extremely low odour thresholds, even when present in very low concentrations, can be important odorants (Jelen and Gracka, 2016). Gas chromatography-olfactometry (GC-O) is the main approach used to study the impact of volatile compounds on sensory perception.

Some analytes with sensory significance present in a food are at low concentrations, therefore they require isolation and concentration from the food prior to analysis (Reineccius and Peterson, 2013). The presence of trace quantities of odour components complicates the extraction/concentration process, to the extent that methods based on volatility, solubility and the use of organic solvents will also extract water, lipids, proteins and carbohydrates, respectively that may necessitate further processing prior to analysis by GC-MS. Odour isolation is difficult because volatiles have a very low molecular mass (< 300 Da) and belong to a large number of different chemical classes (Reineccius and Peterson, 2013). In relation to the different volatile chemical classes identified in these seaweeds, the most abundant were, in decreasing order, hydrocarbons, ketones, aldehydes, alcohols, esters, halogenated compounds, carboxylic acids, furans, phenols, sulphur compounds, pyrazines, pyridines, amines and some miscellaneous compounds.

Terpenes are potentially important odour compounds, where sesquiterpene hydrocarbons have lower impact than monoterpenes due to their higher detection thresholds (Guichard and Salles, 2016). Hydrocarbons are not always relevant to the aroma because of their high odour detection thresholds, but compounds with high odour thresholds can also play an essential role in food aroma when present at high concentrations (Czerny et al., 2011). Ketones are potentially important odour compounds due to their abundance and odour activity. Ketones, such as β-ionone and 6-methyl-5-hepten-2-one, are potent odorant in seafood contributing to the aroma composition of seaweeds (Höckelmann and Jüttner, 2005). The chain length of aldehydes mostly affects odour thresholds and odour properties. Aldehydes with low molecular weight (< 150 Da) tend to be associated with unpleasant odours, and those with higher molecular weights tend to have sweet, fruity odours (Jelen and Gracka, 2016). Aldehydes are important aroma compounds because of their low odour threshold values (Giri et al., 2010). Carbonyl compounds and aldehydes are responsible for various odorant notes such as the nutty alcoholic odour of acetaldehyde (Seo et al., 2012) and the almond odour of benzaldehyde (Guichard and Salles, 2016). Branched-chain alcohols are known to have low odour threshold values, therefore may significantly contribute to odour (Giri et al., 2010). As the ability of alcohols to dissolve in water decreases the odour threshold increases. Therefore unsaturated alcohols have a greater impact on food flavour than saturated alcohols (Jelen and Gracka, 2016). Halogenated compounds are mainly isolated from red and brown seaweeds (Yu et al., 2014). Halogenated compounds substituted with a halogen moiety at the ortho position interact strongly with human odour receptors, resulting in high odour activities (Strube et al., 2014). Aryl halides are fairly pleasant smelling liquids, but arylmethyl (benzylic) halides are irritating to nasal passages (Robert and Caserio, 1977). Acids may increase and enhance food characteristic odour profiles. The strength of odour decreases with increasing acid chain length. Simple acids, with less than six carbon atoms, have high odour thresholds, while long chain acids, with 12 or more carbon atoms, are odourless. Unsaturated acids generally have sharper and stronger odours than saturated ones (Jelen and Gracka, 2016). Esters present mainly fruity notes (e.g. apple odour of butyl acetate) and unsaturated esters have lower thresholds than saturated ones (Guichard and Salles,

2016). Furans consist of a family of important chemicals in the formation of crab flavour (Gu et al., 2013a). Lipoxygenase-catalysed oxidation of linoleic acid can produce 2-pentyl furan which has been associated with 'earthy', 'beany' odour notes (Vara-Ubol et al., 2004). Sulphur compounds are present in very low amounts and possess very low odour thresholds. They are present mainly in brassica vegetables as, for example, dimethyl sulphide responsible for cabbage odour (Jelen and Gracka, 2016). Many pyrazines have intensive odours and very low odour thresholds. Therefore, these compounds contribute to many odours and flavours (Müller and Rappert, 2010). Heterocycles, in the pyridines group, are the main compounds of caramel odour (Paravisini et al., 2015). Amines have a strong ammoniac-like odour. Amines are formed from Strecker degradation products, and their odour depend on the pH value (Guichard and Salles, 2016). Phenolic compounds contribute to the flavour of many foods (Delgado et al., 2015).

The physicochemical properties of these compounds play a major role in sensory perception. Odour thresholds for particular compounds published in the literature can vary extensively and thus it is difficult to estimate an average odour threshold value based on literature data alone (Jelen and Gracka, 2016) (Table 2.1).

Table 2.1. Volatile compounds found in four brown and two red species of edible seaweed.

		Laminaria **spp.**					
	Himanthalia elongata	***Laminaria* spp.**	***Laminaria ochroleuca***	***Undaria pinnatifida***	***Palmaria palmata***	***Porphyra umbilicalis***	
Compound							**Aroma threshold**
Hydrocarbons							
Butane [c]		✓		✓			0.421-5,048 ppm [g]
Hexane [c]		✓		✓			30-248 ppm [g]
Octane [a]	✓		✓	✓	✓	✓	0.66-235 ppm [g]
Nonane [a]	✓		✓	✓	✓	✓	2.3-21 ppm [g]
Undecane [e]				✓			
Dodecane [e]				✓			
Tetradecane [e]				✓			
Pentadecane [a, e]	✓		✓	✓	✓	✓	
Hexadecane [e]				✓			
Heptadecane [a, e]	✓			✓	✓	✓	
Octadecane [e]				✓			
6-Methylpentadecane [e]				✓			
2-Methylheptane [a]	✓		✓	✓	✓	✓	

4-Methylheptane [a]	✓	✓	✓	✓	✓
4-Methyloctane [a]	✓	✓	✓	✓	✓
2-Methylnonane [a]	✓	✓	✓	✓	✓
4-Methyldecane [a]	✓	✓	✓	✓	✓
5-Methyldecane [a]	✓	✓	✓	✓	✓
2-Methylundecane [a]	✓	✓	✓	✓	✓
3-Methylundecane [a]	✓	✓	✓	✓	✓
4-Ethyldecane [a]	✓	✓	✓	✓	✓
2,4-Dimethylhexane [a]	✓	✓	✓	✓	✓
2,4-Dimethylheptane [a]	✓	✓	✓	✓	✓
2,7-Dimethyloctane [a]	✓		✓	✓	✓
2,5-Dimethylnonane [a]	✓	✓	✓	✓	
3,7-Dimethyldecane [a]	✓	✓	✓	✓	✓
2,4-Dimethylundecane [a]	✓	✓	✓	✓	✓
2,6-Dimethylundecane [a]	✓	✓	✓	✓	✓
4,4-Dimethylundecane [a]	✓	✓	✓	✓	✓
4,8-Dimethylundecane [a]	✓	✓	✓	✓	✓
4,6-Dimethyldodecane [a]	✓		✓	✓	✓
2,4,6-Trimethyloctane [a]	✓		✓	✓	

2,6,10-Trimethyl-tetradecane [e]				✓		
Cyclopentane, iodo [c]		✓				
Cyclododecane [b, c]	✓			✓		
Dichloromethane [c]		✓		✓		
Trichloromethane [c]		✓				
(E)-2-Octene [a]	✓		✓	✓	✓	✓
2,4-Dimethyl-1-heptene [a]	✓		✓	✓	✓	✓
1,4-Octadiene [a]	✓		✓	✓	✓	✓
1,3-(*E*)-5-(*Z*)-Octatriene [f]					✓	
1,3-Cyclooctadiene [a]	✓		✓	✓	✓	✓
Trimethyl-1,3-cyclopentadiene [f]					✓	
1,5-Heptadien-3-yne [e]				✓		
2-Methyl-1-hexen-3-yne [e]				✓		
3-Ethyl-2-methyl-1,3-hexadiene [a]	✓		✓	✓	✓	✓
3-Ethylidene-1-methylcyclopentene [f]					✓	
2-(Chloromethyl)-1-butene [c]		✓				
1,3-Dimethylbenzene [a]	✓			✓	✓	✓
1,2,3-Trimethylbenzene [a]	✓			✓	✓	✓

1,2,4-Trimethylbenzene [f]					✓		
Ketones							
2-Propanone [a]	✓		✓	✓	✓	✓	40-476 ppm [h, †]
2-Butanone [a]	✓		✓	✓	✓	✓	n/a [h]
2-Pentanone [a]	✓		✓	✓	✓	✓	70 ppb [h, j]
3-Hexanone [a]	✓		✓	✓	✓	✓	41-81 ppb [h, †]
2-Heptanone [a]	✓		✓	✓	✓	✓	1 ppb-1.33 ppm [h, †] 2.66-3.73 ppm [h, ††]
2-Octanone [a]	✓		✓	✓	✓	✓	41-62 ppb [h, †]
3-Octanone [d]		✓			✓		21-50 ppb [h, †]
1-Penten-3-one [c]				✓			1-13 ppb [h, †]
3-Penten-2-one [a]	✓		✓	✓	✓	✓	1.5 ppb [h, †]
1-Octen-3-one [a]	✓			✓	✓	✓	0.05-4 ppb [h, †]
3-Octen-2-one [a]	✓			✓	✓	✓	n/a [h]
2,3-Butanedione [a]	✓		✓	✓	✓	✓	1-8.6 ppb [i]
2,5-Octadione [a]	✓		✓	✓	✓	✓	
(Z,E)-3,5-Octadien-2-one [a]	✓		✓	✓	✓	✓	
(E,E)-3,5-Octadien-2-one [a, e]	✓		✓	✓	✓	✓	0.15 ppm [h, †]

5,9-Undecadien-2-one, 6, 10-dimethyl-, (z) [e]			✓			60 ppb - 6.4 ppm [h, †]
3,5-Dimethyl-2,7-octadione [a]	✓	✓	✓	✓	✓	
1,3-Cyclohexanedione,2-(2-propenyl)- [e]			✓			
4,4-Dimethyl-2-cyclohexen-1-one [e]			✓			
2(1H)-Naphthalenone, octahydro-8a-methyl-, trans- [e]			✓			
6-Methyl-3,5-heptadien-2-one [a]	✓	✓	✓	✓	✓	375 ppb [h, †]
6-Methyl-5-hepten-2-one [a]	✓	✓	✓	✓	✓	50 ppb [h, †]
1-Hydroxy-2-propanone [a]	✓		✓	✓	✓	
3-Hydroxy-2-butanone [a]	✓	✓	✓	✓		
2,2,6-Trimethylcyclohexanone [a, e]	✓		✓	✓	✓	100 ppb [h, †] 310 ppb [h, ††]
3,5,5-Trimethyl-2-cyclohexen-1-one [a]	✓	✓	✓	✓	✓	
2-Propyl-2-cyclohexenone [a]			✓	✓	✓	
γ-Valerolactone [a]	✓	✓	✓	✓	✓	n/a [h]
γ-Butyrolactone [a]	✓	✓	✓	✓	✓	20-50 ppm [h, †]
1-Phenylethanone [a]	✓	✓	✓	✓	✓	170 ppb [h, †] 2.9 ppm [h, ††]

γ-Hexalactone [a]	✓		✓	✓	✓	✓	1.6 ppm [h, †]
5-Ethyl-2(5H)-furanone [a]	✓		✓	✓	✓	✓	
Isophorone [e]				✓			0.2 ppm [h,j]
4-Ketoisophorone [a]	✓		✓	✓	✓	✓	n/a [h]
α-Ionone [a]				✓	✓	✓	0.6-10 ppb [h, †]
β-Ionone [a, e]	✓		✓	✓	✓	✓	0.007-205 ppb [h, †]
Trans-β-Ionone [a]	✓		✓	✓	✓	✓	
7,8-Epoxy-α-ionone [a]	✓				✓	✓	
1-(4-Acetylphenyl)-ethanone [a]	✓		✓	✓	✓	✓	
Methylethylmaleimide [a]			✓	✓	✓	✓	
Dihydroactinidiolide [a]	✓		✓	✓	✓	✓	
Aldehydes							
Ethanal [a]	✓		✓	✓	✓	✓	0.7-200 ppb [h, †] 27-380 ppb [h, ††]
Propanal [a]	✓		✓	✓	✓	✓	9.5-35 ppb [h, †]
Butanal [a, c]	✓	✓	✓	✓	✓	✓	19-37 ppb [h, †] 11-27 ppb [h, ††]
Pentanal [a, d]	✓	✓	✓	✓	✓	✓	12-100 ppb [h, †]
Hexanal [a, c, d, e]	✓	✓	✓	✓	✓	✓	4.1-22.8 ppb [h, †]

							400 ppb [h, ††]
Heptanal [a, d, e]	✓	✓	✓	✓	✓	✓	3-60 ppb [h, †]
Octanal [a, c, e]	✓	✓	✓	✓	✓	✓	1.4-6.4 ppb [h, †]
Nonanal [a, c, d, e]	✓	✓	✓	✓	✓	✓	1-8 ppb [h, †]
Decanal [c]		✓		✓		✓	0.1-6 ppb [h, †] 9 ppb [h, ††]
Undecanal [c]		✓					0.4-100 ppb [h, †]
Dodecanal [c]		✓					0.5-1.5 ppb [h, †]
(E)-2-Butenal [a]	✓		✓	✓	✓	✓	525 ppb [i, †]
(E)-2-Pentenal [a, c, e]		✓	✓	✓	✓	✓	1.5 ppb [h, †]
(E)-2-Hexenal [a, d, e]	✓	✓	✓	✓	✓	✓	17 ppb - 10 ppm [i, †]
(E)-2-Heptenal [a, e]	✓	✓	✓	✓	✓	✓	13-51 ppb [†]
(Z)-4-Heptenal [a]	✓		✓	✓	✓	✓	0.4 ppb [h, †]
(E)-2-Octenal [a, d, e]	✓	✓	✓	✓	✓	✓	3-4 ppb [h, †]
(E)-2-Nonenal [d, e]		✓		✓			0.1 ppb [h, †]
(E)-2-Decenal [d, e]		✓		✓			1 ppb [h, †]
(E,E)-2,4-Heptadienal [a, c]	✓		✓	✓	✓	✓	n/a [h]
(Z,E)-2,4-Heptadienal [a]	✓		✓	✓	✓	✓	3.6 ppm [i, †]
(E,E)-2,6-Nonadienal [a]	✓		✓	✓	✓	✓	0.09 ppb [h, †]

(E,Z)-2,6-Nonadienal [e]				✓			
(E,E)-2,4-Decadienal [e]				✓			0.07-10 ppb [h, †]
(3Z)-3-Hexenal [c]				✓			0.25 ppb [h, †]
5-Hexenal [c]		✓					
2-Methylbutanal [a]			✓	✓	✓	✓	0.15-140 ppb [i, †]
3-Methylbutanal [a, d]	✓	✓	✓	✓	✓	✓	12 ppb [i, †]
(E)-2-Methyl-2-butenal [a]			✓	✓	✓	✓	500 ppb [h, †]
2-Methyl-2-propenal [a]					✓	✓	
2-Methyl-2-pentenal [a]				✓	✓	✓	290 ppb [h, ††]
2,2-Dimethylpropanal [f]					✓		
Benzaldehyde [a]	✓		✓	✓	✓	✓	100 ppb -4.6 ppm [h, †] 330 ppb -4.1 ppm [h, ††]
4-Ethylbenzaldehyde [a]	✓		✓	✓	✓	✓	13 ppb [h, †]; 40 ppb [h, ††]
4-Propylbenzaldehyde [a]	✓		✓	✓	✓	✓	
β-Cyclocitral [a, e]	✓		✓	✓	✓	✓	
Safranal [a]	✓		✓	✓	✓	✓	
Alcohols							
Ethanol [c]		✓		✓			8-900 ppb [h, †]
1-Propanol [c]		✓		✓	✓	✓	3.2-7500 ppm [i]

1-Butanol [d]		✓					500 ppb - 509 ppm [h, †]
1-Pentanol [a]	✓		✓	✓			32 mg/L liquid [i]
3-Pentanol [a]	✓		✓	✓	✓	✓	
1-Hexanol [a, c]	✓	✓	✓	✓	✓	✓	0.01-5.2 ppm [i]
1-Octanol [a]	✓		✓	✓	✓	✓	42-480 ppb [h, †]
3-Decanol [e]				✓			79-410 ppb [h, †]
1-Penten-3-ol [a, c, d]	✓	✓	✓	✓	✓	✓	400 ppb [h, †]
(E)-2-Penten-1-ol [a]	✓		✓	✓	✓	✓	
(Z)-2-Penten-1-ol [a, c]	✓	✓	✓	✓	✓	✓	
3-Hexen-1-ol [c]		✓		✓			70 ppb [h, †]
5-Hexen-1-ol [c]				✓			
(2E)-2-Hexen-1-ol [c]				✓			
1-Octen-3-ol [a, c, d]	✓	✓	✓	✓	✓	✓	14 ppb [h, †]; 25 ppb [h, ††]
2-Octen-1-ol [a]	✓		✓	✓	✓	✓	40-840 ppb [h, †] 100 ppb [h, ††]
(E)-2-Octen-1-ol [d]		✓					n/a [h]
(E)-2-Nonen-1-ol [d]		✓					130 ppb [h, †]
2,3-Butanediol [a]				✓			
(Z)-1,5-Octadien-3-ol [a]	✓		✓	✓	✓	✓	

1,7-Octadien-3-ol [a]	✓	✓	✓			
3,5-Octadien-2-ol [a]	✓	✓	✓	✓		
3-Methylbutanol [f]				✓		250 ppb - 4.1 ppm [h, †]
2-Methyl-3-pentanol [a]	✓	✓	✓	✓		
(E)-2-Ethyl-1-hexanol [a]	✓	✓	✓	✓		n/a [h]
(Z)-2-Ethyl-1-hexanol [a]	✓					n/a [h]
2-(2-Methoxypropoxy)-1-propanol [a]	✓	✓	✓	✓	✓	
1-(2-Methoxypropoxy)-2-propanol [a]	✓	✓	✓	✓	✓	
1-(2-Methoxy-1-methylethoxy)-2-propanol [a]	✓	✓	✓	✓	✓	
1-Ethoxy-2-propanol [a]	✓	✓	✓	✓	✓	
Cyclooctanol [a]	✓		✓	✓	✓	
1,2-Dimethyl-cyclohexanol [a]	✓	✓	✓	✓	✓	
2,4-Dimethyl-cyclohexanol [e]			✓			
4,4-Dimethyl-cyclohex-2-en-1-ol [e]			✓			
Benzenemethanol [a]	✓	✓	✓	✓	✓	1.2-1000 ppb – 10-1000 ppm [h, †]
Halogenated compounds						
Iodo-methane [a]		✓		✓		

Iodo-ethane [a]			✓		✓		
2-Iodo-propane [a, c]		✓	✓	✓	✓		
1-Iodo-pentane [a, c]	✓		✓	✓	✓		
1-Iodo-octane [d]		✓	✓				
2-Fluoroprop-1-ene [f]					✓		
Chlorobenzene [f]					✓		0.21 ppm [i]
Dichloromethane [f]					✓		210-214 ppm [i]
Tribromomethane [d]		✓			✓		4.8×10^8 molecules/cc [i]
Trichloromethane [d]		✓			✓		
5-Chloro-1-pentene [a]			✓				
Carboxylic acids							
Ethanoic acid [a]	✓		✓	✓	✓	✓	10-522 ppm [†] 60 ppm [h, ††]
Butanoic acid [a]	✓		✓	✓	✓	✓	240 ppb - 4.8 ppm [h, †]
Hexanoic acid [a]	✓		✓	✓	✓	✓	93 ppb - 10 ppm [h, †]
Heptanoic acid [a]	✓		✓	✓	✓	✓	640 ppb - 10.4 ppm [h, †]
Octanoic acid [a]	✓		✓	✓	✓	✓	910 ppb - 19 ppm [h, †]
2-Methylpropanoic acid [a]	✓		✓	✓		✓	10 ppb - 9.5 ppm [h, †]
3-Methylbutanoic acid [a]	✓		✓	✓	✓	✓	

Esters						
Acetic acid ethyl ester [c]			✓			5 ppb – 5 ppm [h, †]
Lactic acid ethyl ester [a]			✓			50-250 ppm [h, †]
Octyl benzoate [e]			✓			
Propanoic acid, 2-methyl-,1-(1,1-dimethylethyl)-2-methyl-1,3,-propanediyl ester [e]			✓			
Hexanedioic acid, bis(2-methylpropyl) ester [e]			✓			n/a [h]
Dodecanoic acid, 1-methyl ester (isopropyl laurate) [e]			✓			n/a [h]
Pentanedioic acid, dibutylester [c]		✓	✓			
Phthalic acid, diisobutylester [e]			✓			
1,2,3-Propanetriol triethanoate [a]			✓			n/a [h]
1,2-Benzenedicarboxylic acid, diethyl ester [a]	✓		✓	✓	✓	
Furans						
2-Ethylfuran [a, c, e]	✓	✓	✓	✓	✓	n/a [h]
2-Methylfuran [a]	✓	✓	✓	✓	✓	27 ppm [i]
2-Pentylfuran [a]	✓	✓	✓	✓	✓	6 ppb [h, †]
cis-2-(2-Pentenyl)furan [a]	✓	✓	✓	✓	✓	

Sulfur compounds						
Dimethyl sulfoxide [a]	✓	✓	✓	✓	✓	
Dimethyl sulfide [a]	✓	✓	✓	✓	✓	0.3-10 ppb [h, †]
Phenols						
Phenol,2,4-bis (1,1-dimethylethyl)- [b]	✓					
Phenol,2,6-bis (1,1-dimethylethyl)-4-methyl [b]	✓					
Amines						
N,N-Dimethyl-methanamine [a]	✓	✓		✓	✓	0.3-0.8 ppb [h, †] 500 ppb [h, ††]
Pyrazines						
2,6-Dimethylpyrazine [a]		✓	✓			400-1500 ppb [h, †]
2,3,5,6-Tetramethylpyrazine [a]				✓		1-10 ppm [h, †]
Pyridines						
2-ethylpyridine [a]			✓	✓		4-22 ppm [h, †]
Miscellaneous						
1-Acetyl-2,2-dimethylcyclobutane [a]		✓				
1,2-Benzisothiazole [e]			✓			

1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl- [e]			✓		
1-Methoxy-4-(1'-methylethyl)cyclohexa-1,4-diene [a]	✓		✓	✓	✓
2,5-Dimethylhexane-2,5-dihydroperoxide [e]			✓		
2,7-Epoxy-megastigma-4,8-diene [a]	✓		✓	✓	
Stigmasta-5,24(28)dien-3-ol (Fucosterol) [a]	✓				

[a] (López-Pérez et al., 2017); [b] (Merichel Plaza et al., 2010); [c] (Ferraces Casais et al., 2013); [d] (Takahashi et al., 2002); [e] (Balbas et al., 2015); [f] (Le Pape et al., 2004); [g] (Murnane et al., 2013), diluent was assumed to be air, unless specified otherwise in the paper; [h] (Burdock, 2005), representative values in distilled water unless otherwise designated; [i] (American Society for Testing and Materials, 1978), representative values in water unless otherwise designated; [†] Detection; [††] Recognition; ✓ Identified/Present in the seaweed; Brown species: *Himanthalia elongata*, *Laminaria* spp., *Undaria pinnatifida*, *Laminaria ochroleuca.* Red species: *Palmaria palmata*, *Porphyra umbilicalis*.

2.4. Key volatiles identified in commercially important seaweed species

2.4.1. Extraction and analytical methods

The major volatile components identified in commercially important seaweeds that likely have an impact on sensory perception are briefly summarized. The extraction/concentration conditions and polarity of the gas chromatography (GC) columns and methods of detection varied between studies (Beauchêne et al., 2000; Gressler et al., 2010; Le Pape et al., 2004; Michalak et al., 2015; Roohinejad et al., 2017; Takahashi et al., 2002).

López-Pérez et al. (López-Pérez et al., 2017) used HS-SPME GC-MS with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber to identify volatiles in dried samples of five of the six seaweeds reviewed in this study (*H. elongata*, *U. pinnatifida*, *L. ochroleuca*, *P. umbilicalis* and *P. palmata*). These authors optimized the extraction/concentration conditions (equilibration time, extraction time and temperature and sample size to headspace volume ratio) and were able to identify a large number of volatiles compounds. They used a polyethylene glycol highly polar column and a single quadrupole (Q) mass spectrometer (MS) over the mass range of 35 to 300 m/z.

Balbas et al. (Balbas et al., 2015) also used HS-SPME with a DVB/CAR/PDMS fiber, but incubated 0.5 g of dried sample (*U. pinnatifida*) for 30 min at 35 °C in a 10 ml headspace vial with 2 ml deionized water. The column was a 5 % (1,4-bis(dimethylsiloxy)phenylene/95 % dimethyl polysiloxane low polarity column. The mass scan of the Q MS was from 48 to 400 m/z.

Ferraces-Cascais et al. (Ferraces Casais et al., 2013) used a purge and trap (sorbent not provided) to extract volatiles from fresh samples of two types of macroalgae (*Laminaria* spp. and *U. pinnatifida*) by a 5 % diphenylpolysiloxane and 95 % dimethyl-polysiloxane low polarity column with a Q MS, scanning a mass range of m/z 35–400. Le Pape et al. (Le Pape et al., 2004) also used purge and trap (Tenax TA 80-100 mesh) at 25 °C; and a polyethylene glycol highly polar column and a single quadrupole MS over the mass range of 33 to 300 m/z.

Takahashi et al. (Takahashi et al., 2002) used a capillary column (no specifications) to extract volatiles from *Laminaria* spp. Plaza et al. (Merichel Plaza et al., 2010) used simultaneous distillation and extraction under reduced pressure and an accelerated solvent extractor with hexane, ethanol and water as solvents to extract volatiles from *H. elongata*. The GC column was a fused silica capillary column coated with a 0.25 µm layer of SE-54 (94 % methyl, 5 % phenyl, 1 % vinyl polysilicone) low polarity, coupled to a quadrupole MS using a mass interval ranging from 35 to 450 m/z. These authors also optimized extraction conditions in terms of solvents and temperature.

2.4.2. Volatile compounds of seaweed species

2.4.2.1. Brown seaweed species

2.4.2.1.1. Himanthalia elongata (Sea spaghetti)

Himanthalia elongata or 'sea spaghetti' belongs to the Phaeophyta or brown macroalgae (Garcia Vaquero et al., 2017). This edible brown seaweed is commonly harvested along the European side of the Atlantic Ocean (Rajauria et al., 2017). It has a history of safe use and acceptability in cooking and was previously used to add a beefy or nutty-like flavour to dishes (Garcia Vaquero et al., 2017). Indeed, beef patty formulations containing *H. elongata* seaweed were improved in texture and mouth-feel preserving their sensory quality, and were of the most overall accepted (Cox and Abu-Ghannam, 2013).

López-Pérez et al. (López-Pérez et al., 2017) found 34 hydrocarbons in *H. elongata*, but only 30 could be positively identified. The most abundant branched-chain hydrocarbon was 4-ethyldecane and the most abundant linear saturated hydrocarbon was octane. Plaza et al. (Merichel Plaza et al., 2010) only identified one hydrocarbon, cyclododecane, in *H. elongata*. López-Pérez et al. (López-Pérez et al., 2017), identified 31 ketones; 2-propanone (acetone) predominated among linear saturated ketones, 3,5-octadien-2-one among linear unsaturated ketones and 3-

hydroxy-2-butanone among branched-chain unsaturated ketones. *H. elongata* had the lowest concentration of volatile aldehydes of the seven seaweeds included in their study (López-Pérez et al., 2017). These same authors identified 22 aldehydes, with hexanal as the main linear saturated aldehyde, and (E)-2-hexenal as the main linear unsaturated aldehyde and benzaldehyde the main aromatic aldehyde. They also identified 22 volatile alcohols, with 1-(2-methoxypropoxy)-2-propanol the most abundant branched-chain alcohol, the most abundant linear unsaturated alcohols were 1-octen-3-ol and 1-penten-3-ol, with 1-pentanol as the most abundant linear saturated one. Only one halogenated compound (1-iodopentane), one ester (1,2-benzenedicarboxylic acid diethyl ester), and one amine (dimethylmethanamine) were detected in *H. elongata*. Seven carboxylic acids were also identified, the most abundant linear acid was ethanoic (acetic) acid, whereas the most abundant branched-chain acid was 3-methylbutanoate. Four furans were also detected; 2-methylfuran, cis-2-(2-pentenyl)furan, 2-ethyl-furan and 2-pentyl-furan, with the latter two being the most predominant. Two sulphur compounds, dimethyl sulphide and dimethyl sulfoxide were also detected as were two dienes, 7-epoxy-megastigma-4,8-diene and 1-methoxy-4-(1'-methylethyl)cyclohexa-1,4-diene.

Plaza et al. (Merichel Plaza et al., 2010) also identified two phenols; 2,4-bis-(1,1-dimethylethyl)- and phenol,2,6-bis-(1,1-dimethylethyl)-4-methyl; two fatty acids, tetradecanoic (myristic) and hexadecanoic (palmitic) acid; stigmasta-5,24(28)dien-3-ol (fucosterol) and cyclododecane in *H. elongata.* The volatile compounds identified in *H. elongata* can be found in Table 2.1.

2.4.2.1.2. Laminaria spp.

Laminaria spp. are members of brown algae, the most important seaweed cultured in the Pacific Ocean from the economical point of view (Cai et al., 2014). The yearly output of this seaweed is approximately 15 million tons in dry weight (Lu et al., 2014). At present, it is valued mainly as food and widely consumed in East Asia (Jia et al., 2014).

Ferraces-Casais et al. (Ferraces Casais et al., 2013) identified 26 volatile compounds in *Laminaria* spp.; six were hydrocarbons, with butane the most abundant. The hydrocarbons constituted the second most abundant family of volatiles present. Alcohols were the most important class of volatile compounds identified (29 % of compounds identified). Seven alcohols were identified with 1-penten-3-ol as the most predominant one. Takahashi et al. (Takahashi et al., 2002) also identified 1-penten-3-ol and 1-butanol in Kombu. Ten aldehydes were identified with hexanal the most abundant. Takahashi et al. (Takahashi et al., 2002) also identified pentanal, 2-(E)-hexenal, hexanal, heptanal, nonanal in Kombu. Pentanedioic acid, dibutylester was the only ester identified by Ferraces-Casais et al. (Ferraces Casais et al., 2013). Six halogenated compounds were also identified, among them 1-iodo-pentane and 2-iodo-propane. Takahashi et al. (Takahashi et al., 2002) found in addition iodopentane, trichloromethane and tribromomethane. The low amount of chemical classes identified is more likely due to the extraction method (purge and trap) employed in relation to the polarity of the compounds present. Takahashi et al. (Takahashi et al., 2002) studied volatile compounds of dried Kombu (*Laminaria* spp.) by SDE and DHS. The 49 volatiles of Kombu analysed by DHS were almost the same as identified using SDE, except trichloromethane and tribromomethane were not detected by DHS, and 3-methylbutanal, (E)-2-octenal, (E)-2-decenal (aldehydes); (E)-2-octen-1-ol, (E)-2-nonen-1-ol (alcohols) and 1-iodo-octane (halogenated compound) were only present by DHS. Only 1-iodooctane had an odour from the 4 kinds of iodides detected. The volatile compounds identified in *Laminaria* spp. can be found in Table 2.1.

Laminaria ochroleuca belongs to brown algae (Filum Ochrophyta, Class Phaeophyceae of the family of Laminariaceae). *L. ochroleuca* specimens grow in subtidal areas (from Morocco to southern UK and in the Mediterranean), and are abundant in the Atlantic coast of Cadiz (Southern Spain) (Abdala Díaz et al., 2019).

López-Pérez et al. (López-Pérez et al., 2017) identified 128 volatile compounds in *L. ochroleuca*. The numbers varied from 30 hydrocarbons to 26 aldehydes, 7 carboxylic acids, to none esters. Volatile ketones, alcohols and furans reached their lowest levels in *L. ochroleuca* compared to the other species studied. Twenty-seven, 20 and 4 compounds of each class were identified, respectively. Total volatile

halogenated compounds reached their highest in *L. ochroleuca*, which contained 6 halogenated compounds. The most abundant one was iodo-methane followed by 2- and 1-iodopentane. Other volatile compounds detected in *L. ochroleuca* were 1-acetyl-2,2-dimethylcyclobutane, 1-methoxy-4-(1'-methylethyl)cyclohexa-1,4-diene and 2,7-epoxy-megastigma-4,8-diene. A summary of the volatile compounds identified in *L. ochroleuca* can be found in Table 2.1.

2.4.2.1.3. Undaria pinnatifida (Wakame)

Undaria pinnatifida or Wakame is a type of brown seaweed that normally grows in the seas of Japan, Korea and New Zealand, but is also commonly found along Western European coasts as an invasive species (Epstein and Smale, 2017). Its structure can be divided into the blade (lamina), midrib, sporophyll and root-like formations (haptera) (Fung et al., 2013). Wakame is one of the most important global economic marine algae and has a long-standing tradition as food for human consumption (Li et al., 2015).

López-Pérez et al. (López-Pérez et al., 2017) identified 35 hydrocarbons in *U. pinnatifida*, where 4-ethyldecane, pentadecane and 2-methylnonane were the most abundant. The number of hydrocarbons in the volatile fraction of *U. pinnatifida* found in other studies varied from 10 alkanes in Balbas et al. (Balbas et al., 2015) to two hydrocarbons in Ferraces-Casais et al. (Ferraces Casais et al., 2013), however differences are likely related to the choice of extraction/concentration methods and operating conditions. Hexadecane and heptadecane (Balbas et al., 2015) and butane (Ferraces Casais et al., 2013) were the most abundant hydrocarbons.

López-Pérez et al. (López-Pérez et al., 2017) also found 34 ketones; with (Z,E)-3,5-octadien-2-one, and 2-propanone and 2-butanone as predominant ones among linear unsaturated and linear saturated ketones, respectively. Balbas et al. (Balbas et al., 2015) identified eight volatile ketones in *U. pinnatifida*, with both (E,E)-3,5-octadien-2-one and β-ionone the most abundant ones, whereas Ferraces-Casais et al. (Ferraces Casais et al., 2013) only identified 1-penten-3-one.

Benzaldehyde was the major aromatic aldehyde, 2-hexenal the major linear unsaturated aldehyde and hexanal was by far the major linear saturated aldehyde (López-Pérez et al., 2017). Thirteen (Balbas et al., 2015) and seven (Ferraces Casais et al., 2013) aldehydes were observed in dried *U. pinnatifida*. Hexanal and 2-heptenal were the most abundant aldehydes found by Balbas et al. (Balbas et al., 2015), while hexanal was the most abundant one in the study by Ferraces-Casais et al. (Ferraces Casais et al., 2013).

López-Pérez et al. (López-Pérez et al., 2017) identified 22 alcohols, the most abundant branched-chain alcohol was 1-(2-methoxypropoxy)-2-propanol, while 1-octen-3-ol and 1-penten-3-ol predominated among linear unsaturated alcohols. There was also a high concentration of (Z)-1,5-octadien-3-ol. Ferraces-Casais et al. (Ferraces Casais et al., 2013) reported nine alcohols while Balbas et al. (Balbas et al., 2015) only identified three. The main alcohols were 1-penten-3-ol (Ferraces Casais et al., 2013) and 3-decanol (Balbas et al., 2015).

U. pinnatifida also contained six halogenated compounds and seven carboxylic acids in López-Pérez et al. (López-Pérez et al., 2017) study, with ethanoic (acetic) acid the most plentiful one. *U. pinnatifida* also had an 1-iodo-pentane as the only halogenated compound (Ferraces Casais et al., 2013). Only Balbas et al. (Balbas et al., 2015) perceived carboxylic acids, 2-methylpentanoic and 9- hexadecenoic acids.

1,2-benzenedicarboxylic acid diethyl ester appeared in greater amounts in all seaweed samples than *U. pinnatifida*, while lactic acid ethyl ester and 1,2,3-propanetriol triethanoate were only identified in *U. pinnatifida* (López-Pérez et al., 2017). Ferraces-Casais et al. (Ferraces Casais et al., 2013) found two esters in *U. pinnatifida*, with the most abundant dibutyl ester of pentanedioic acid, while Balbas et al. 2015 (Balbas et al., 2015) found five, with hexanedioic acid, bis(2-methylpropyl) as the predominant ester.

López-Pérez et al. (López-Pérez et al., 2017) identified four furans, with the greatest concentration of 2-ethyl-furan and 2-pentyl-furan. The volatile fraction of *U. pinnatifida* in other studies also included 2-ethyl-furan (Balbas et al., 2015; Ferraces Casais et al., 2013).

A low abundance of sulphur compounds was evident in *U. pinnatifida*. The two sulphur compounds identified were dimethyl sulphide and dimethyl sulfoxide (López-

Pérez et al., 2017). One pyrazine, 2,6-dimethylpyrazine; and one pyridine, 2-ethylpyridine were also detected (López-Pérez et al., 2017). Two other volatile compounds were also detected; 2,7-epoxy-megastigma-4,8-diene and 1-methoxy-4-(1'-methylethyl)cyclohexa-1,4-diene (López-Pérez et al., 2017). 1,2-Benzisothiazole, 1,3-cyclopentadiene, 5,5-dimethyl-2-ethyl- and 2,5-dimethylhexane-2,5- were also detected by Balbas et al. (Balbas et al., 2015) in *U. pinnatifida*. A summary of the volatile compounds identified in *U. pinnatifida* can be found in Table 2.1.

2.4.2.2. Red seaweed species

2.4.2.2.1. Palmaria palmata (Dulse)

Palmaria palmata (*Rhodymenia palmata*) is a red alga (Rhodophyta) in the family Palmariaceae. It grows on the northern coasts of Pacific and Atlantic oceans. It is commonly known as 'dulse' (Banskota et al., 2014), and it has recently become popular as a foodstuff (Harnedy et al., 2015). In Ireland especially and many parts of the British Isles, *P. palmata* is widely available and has a strong historical tradition as component of the local diet (Allsopp et al., 2016).

López-Pérez et al. (López-Pérez et al., 2017) identified 31 hydrocarbons in *P. palmata*. Heptadecane was the most abundant linear saturated hydrocarbon and 3-methylundecane the second most abundant hydrocarbon. Le Pape et al. (Le Pape et al., 2004) identified four hydrocarbons, with 3-ethylidene-1-methylcyclopentene as the most abundant one.

Thirty two ketones were identified of which 2-propanone (acetone) and 2-butanone were the most abundant (López-Pérez et al., 2017), while Le Pape et al. (Le Pape et al., 2004) identified only two ketones (2,3-pentanedione and 3-octanone).

P. palmata had the highest concentration of aldehydes in the study by Lopez-Perez et al. (López-Pérez et al., 2017), with 28 aldehydes identified. Propanal was the major linear saturated aldehyde and (E)-2-pentenal the major linear unsaturated aldehyde and benzaldehyde the major aromatic aldehyde. Le Pape et al. (Le Pape et

al., 2004) identified seven aldehydes with octanal and nonanal as the most predominant.

There was also a high concentration of volatile alcohols, corresponding to 20 different compounds (López-Pérez et al., 2017). The most abundant branched-chain alcohol was 1-(2- methoxypropoxy)-2-propanol, while 1-octen-3-ol and 1-penten-3-ol predominated among linear unsaturated alcohols and 1-pentanol among linear saturated alcohols (López-Pérez et al., 2017). Le Pape et al. (Le Pape et al., 2004) identified three alcohols (1-butanol, 1-penten-3-ol and 3-methylbutanol).

P. palmata also contained three halogenated compounds, the most abundant was 1-iodopentane; six carboxylic acids, being ethanoic (acetic) acid the most abundant and one ester (1,2-benzenedicarboxylic acid diethyl ester). *P. palmata* also contained high abundances of dimethyl sulphide and dimethyl sulfoxide and two pyrazines, 2,6-dimethylpyrazine and 2,3,5,6-tetramethylpyrazine were also identified. In dried *P. palmata* samples, other pyrazines; 2,5-diethylpyrazine and 3-ethyl-2,5-dimethylpyrazine were also reported (Michel et al., 1997). The only pyridine and amine detected by López-Pérez et al. (López-Pérez et al., 2017) were 2-ethylpyridine and dimethylmethanamine, respectively, and were the most abundant in *P. palmata* in comparison to the other aldehydes. Le Pape et al. (Le Pape et al., 2004) identified seven halogenated compounds, the most abundant were iodoethane, tribromomethane and chlorobenzene. Neither carboxylic acids nor esters, furans, sulphur compounds, pyrazines or any other volatile compounds were detected by Le Pape et al. (Le Pape et al., 2004) in *P. palmata* samples, but this could be due to the DHS/concentration methodology used.

The volatile compounds identified in *P. palmata* can be found in Table 2.1.

2.4.2.2.2. Porphyra umbilicalis (Nori)

Porphyra umbilicalis (commonly known as Nori) is a macrophytic red alga – Rhodophyta- that belongs to an ancient group of red algae, the Bangiophyceae. It is important ecologically and economically (Kim et al., 2016) and is harvested for

human food, and thrives in the harsh conditions of the upper intertidal zone (Brawley et al., 2017).

López-Pérez et al. (López-Pérez et al., 2017) detected 32 hydrocarbons in *P. umbilicalis* which was the lowest number detected in the seven seaweeds evaluated, four of them were unidentified branched-chain hydrocarbons. The most abundant were 3-methylundecane, 4-methyldecane and 2-methylnonane. These authors identified a considerable number (34) of ketones in *P. umbilicalis*, with (E,E)-3,5-octadien-2-one as the most abundant one. Twenty-seven aldehydes were also detected, with both hexanal and pentanal as the most abundant. The most abundant alcohols were 1-octen-3-ol and 1,2-dimethylcyclohexanol. No halogenated compounds were detected. The authors identified seven carboxylic acids, with both ethanoic (acetic) and hexanoic acids the most abundant and also one ester (1,2-benzenedicarboxylic acid diethyl ester). These authors identified 4 furans in *P. umbilicalis* with 2-ethyl-furan and 2-pentyl-furan as the most abundant and only 2 pyrazines, 2,6-dimethylpyrazine and 2,3,5,6-tetramethylpyrazine. The volatile compounds identified in *P. umbilicalis* can be found in Table 2.1.

2.5. Discussion and future perspectives

Macroalgae are already incorporated as nutritional ingredients into food and drinks, but the search for the ideal macroalgae for a wide range of applications is still ongoing. The addition of small amounts of seaweeds or their extracts to food products opens up new prospects for food processing, meat product formulations included. A major difficulty with seaweeds and similar plants is that biological and chemical characteristics may change during growth, in the period from harvest to consumption, and during processing. These changes pose challenges in identifying key character impact volatile(s) that may influence sensory perception in processed foods to which they have been added.

Specific knowledge gaps exist in these chosen macroalgae species regarding, the effect of different harvesting, storage and processing methods of seaweeds on volatiles; the identification of key volatile compounds that impact sensory perception

and the information on the application of seaweeds in processed foods from a sensory perspective.

Regarding storage, the quality of the product is determined by its stability during storage, among other factors (thickness, hardness, colour and the absence of foreign materials) (Balbas et al., 2015). Ideally samples should be extracted and analysed soon after collection because compounds may change even if frozen (e.g. under nitrogen gas) (Cronin et al., 1995). However, it is often necessary to store samples for analysis later in time, due to common setbacks. In any case, analysis of a few representative samples should be made, whenever possible, soon after collection so qualitative and quantitative changes in chemical composition can be detected. When immediate analysis is impossible, samples should be stored in a manner that minimizes changes in metabolite composition. For this purpose, further research (Cronin et al., 1995; Le Pape et al., 2002) is required.

Regarding the processing, the nature of many secondary metabolites suggests that some may be labile, necessitating the use of methodologies that do not alter the concentration or structure of compounds (Cronin et al., 1995). Some authors have studied the influence of processing methods on the volatile compounds of different seaweeds, and depending on the method of their preparation the volatile content may vary greatly (Ferraces Casais et al., 2013; Takahashi et al., 2002). However, deeper knowledge on this aspect is also limited and merits further investigation.

It is evident that the production of volatile organic compounds by seaweeds is closely related to the physiology of the species, habitats, maturity (Ortiz et al., 2006; Peinado, Girón, et al., 2014), geographical origin and environmental conditions (López-Pérez et al., 2017). Algae are vegetative organisms and must adapt to abiotic stresses (humidity, mineral composition and the sea temperature, light amount and intensity) during their life cycle (Cox and Abu-Ghannam, 2013; Maehre et al., 2014). Therefore, the different compositions and properties of the volatile organic compounds imply different growth conditions of the same species (Gressler et al., 2009).

Furthermore, consumer acceptance of food is closely related to its sensory properties, of which flavour is of prime importance. Some volatile compounds have great impact on the aroma of seaweeds and are considered “character-impact

compounds". The diversity of these volatiles compounds present in seaweeds produce different odours like "seafood", "marine", "fish", "liquorice", "spices", "fatty", "green" and "honey" (Leite Santos and Narain, 2018).

Carboxylic acids are related to odour notes such as spices, honey and liquorice, while esters are related to green odour. Notes like fish, marine, fatty and seafood odour are related to presence of amines and pyridines. When present in higher concentrations, compounds contribute to fruity notes in aroma characteristics of some seaweeds (Van Durme et al., 2013a). Another class of compounds commonly found in the aroma composition of seaweeds are the ketones, such as β-ionone and 6-methyl-5-hepten-2-one, both potent odorants in seafood. The latter ketone results in compounds having more pleasant odours in seaweed and other foods, such as tomato (Leite Santos and Narain, 2018). Furans are evident in some species of seaweed and are important compounds in the formation of crab flavour, as highlighted by Gu et al. study (Gu et al., 2013b). Character-impact compounds also include halogenated compounds containing bromine (e.g. dibromoethane, dibromochloromethane, bromodichloromethane and chloroiodomethane) and iodine (e.g. iodoethane, iodopentane) and isoprene (2-methyl-1-3-butadiene), which contribute to the characteristic aroma of the seaweeds, with aromas like marine, crustacean and green (Lanturnus et al., 1996); and sweet notes (Amsler and Fairhead, 2005), especially in red seaweeds (Broadgate et al., 2004).

The aroma diversity of seaweeds is a result of the variety and concentration range of the aroma compounds. Some of these aroma characteristics may be desirable in some applications but unwanted in others. Therefore, further studies to elucidate which edible seaweeds are more suitable in food and feed formulations are necessary. The detailed identification of volatile compounds is very important to elucidate their aroma properties of the final product (Maruti et al., 2018).

2.6. Conclusion

The production of volatile organic compounds by plants/algae is well known. However, little research has been undertaken on the volatile compounds present in

commercially important brown seaweeds (*Himanthalia elongata*, *Laminaria* spp. *Undaria pinnatifida*, and *Laminaria ochroleuca*) or red seaweeds (*Porphyra umbilicalis* and *Palmaria palmata*).

More than 200 different volatile compounds, belonging to 13 or more chemical groups, were present in the six seaweed species reviewed. There was only a limited amount of information available on *Laminaria* spp.

The major volatile compounds found in seaweeds, in decreasing order, were hydrocarbons, ketones, aldehydes, alcohols, halogen or sulphur-containing compounds, acids, esters, furans, and phenols. Volatile compounds reported in previous works depended, mostly, on the extraction methodology and GC-MS conditions. The impact of volatile compounds on sensory perception is dependent upon abundance and odour threshold.

Extended work needs to be done related to the volatile compounds of seaweeds in connection with their sensory characteristics. Further work is aimed at evaluating the aroma characteristics of seaweeds with the objective of evaluating the influence of flavour and odour notes on acceptability of new seaweed-containing products.

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Chapter 3

Chapter 3. A chemometric approach to characterize the aroma of selected brown and red edible seaweed / extracts

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3.1. Abstract

Information pertaining to the aromatic profile of seaweeds and seaweed extracts can provide evidence regarding their potential suitability as ingredients in processed foods. To date only limited material has been available on the volatile profiles of some seaweed species. Others in this study have not previously been described. The volatile profiles of dried brown (*Himanthalia elongata*, *Undaria pinnatifida*, *Alaria esculenta*) and red (*Porphyra umbilicalis*, *Palmaria palmata*) seaweeds, and a brown seaweed extract (fucoxanthin) from *Laminaria japonica* were investigated using a chemometric approach to collate data from volatile gas chromatography-mass spectrometry (GC-MS), direct sensory aroma evaluation, and gas-chromatography-olfactometry (GC-O) to obtain a better understanding of their volatile profile and sensory perception. More than 100 volatile compounds were identified by static headspace solid phase micro-extraction (HS-SPME) and thermal desorption gas chromatography-mass spectrometry (TD GC-MS). Brown seaweeds were characterized by 'grassy/ herbal/floral', 'fruity', and 'fatty' aromas, red seaweeds by 'green/vegetable', 'mushroom/earthy' and 'sweet/buttery' aromas, and the fucoxanthin extract by 'rancid' and 'nutty' aromas with an overall lower intensity. Heptanal appeared to be a major odour-active compound in all samples. Other volatiles were more characteristic of each individual seaweed: hexanal, (E,Z)-2,6-nonadienal and 2-pentylfuran for *H. elongata*; ethyl butanoate and 2,3-butanedione for *U. pinnatifida*; 6-dimethylpyrazine, (E,Z)-2,6-nonadienal and sulactone for *P. palmata*; 1-octen-3-ol for *P. umbilicalis*, heptanone for *A. esculenta*, and 2-furanmethanol for fucoxanthin. Brown and red seaweeds had distinct sensory properties with individual seaweeds having differing volatiles and odorants. This

study provides additional information that can contribute to the development of products incorporating dried seaweeds/extracts that are more acceptable to the consumer.

3.2. Introduction

Volatile compounds are fundamental for the aroma and odour perception of seaweed (Yamamoto et al., 2014). The volatile compounds of brown and red seaweed have been investigated previously (Gressler et al., 2010b; Hattab et al., 2007; Yu et al., 2010). However, little information is available on species such as *Himanthalia elongata*, *Undaria pinnatifida*, *Alaria esculenta*, *Laminaria japonica*, *Porphyra umbilicalis* and *Palmaria palmata*, which are found in abundance around the coastlines of Western Europe (see www.algaebase.org) (Balbas et al., 2015; Ferraces Casais et al., 2013; Le Pape et al., 2004; López-Pérez et al., 2017; Plaza et al., 2010). From an extensive review of the scientific literature, there appears to be no publications pertaining to the volatile profiles and odour characteristics of the seaweeds *A. esculenta* and *L. japonica*.

Gas chromatography-mass spectrometry (GC-MS) is one of the most powerful analytical methods used in flavour chemistry. Complex mixtures are separated by GC, and the components are then identified by MS (Kameoka, 1986). Many methods exist for the extraction and pre-concentration of analytes in solid samples (Nor Qhairul Izzreen and Vijaya Ratnam, 2011). However, no single extraction method is perfect, as each has a degree of bias, mainly due to issues relating to polarity, molecular weight or vapour pressure (Garicano Vilar et al., 2020b). A definite movement towards automated approaches has occurred due to ease of use, time efficiency, and general effectiveness (Garicano Vilar et al., 2020b). To date, the extraction of volatile compounds from seaweeds has been carried out by dynamic headspace extraction (purge and trap) (Ferraces Casais et al., 2013), distillation-solvent extraction (Kamenarska et al., 2006b) and static headspace solid phase micro-extraction (HS-SPME) (Nor Qhairul Izzreen and Vijaya Ratnam, 2011). In this study, two automated extraction techniques were evaluated: (i) HS-SPME, a static headspace technique

widely used for the identification of volatile organic compounds (VOC) because of its ease of use and wide range of fibers available to target different chemical classes; and (ii) thermal desorption (TD), a dynamic technique that has a much larger sorbent-phase capacity with an enrichment capability.

Gas chromatography-olfactometry (GC-O) uses human assessors in parallel with a detector, such as flame ionization or mass spectrometric (MS) detector, to obtain sensory and chemical responses for aroma compounds, which enable the identification of key odorants that exert the greatest impact on sensory perception (Minteguiaga et al., 2015). GC-O has been extensively applied for the characterization of aroma impact compounds in a variety of matrices, mainly in food-related fields like coffee (Chin et al., 2015), tea (X Chen et al., 2019), meat (Tao et al., 2014) and others (da Rocha et al., 2017). Nevertheless, very few studies (Guo et al., 2019; Isleten Hosoglu, 2018) have utilized GC-O to evaluate seaweeds and/or seaweed extracts. The volatiles in seaweeds produce a range of different odours that could be categorised as 'marine' or 'seafood'. Other odours such as 'fish', 'fatty', 'honey', 'green', 'floral' and 'spicy' have also been detected in various green, red and brown seaweeds (Leite Santos and Narain, 2018; Yamamoto et al., 2014). In this study, HS-SPME GC-O was employed to elucidate the key aroma compounds in five dried edible seaweed species and in an edible fucoxanthin powder extract. An aroma assessment was also undertaken to provide a more expansive profile, because a potential weakness of GC-O is that assessors can only sniff individual or co-eluted volatiles, and may miss any aromas generated from the combination of volatiles and possibly aspects relating to the composition of the product.

The primary objective of this study was to use a chemometric approach to investigate the aroma of five dried edible seaweed species: four brown (one in the form of a powder extract, fucoxanthin), and two red species, to determine their main odour active properties.

3.3. Material and methods

3.3.1. Seaweed material

The edible seaweeds used in this study were four brown seaweed species (*H. elongata*, *U. pinnatifida*, *A. esculenta* and *L. japonica*) and two red species (*P. umbilicalis* and *P. palmata*). *H. elongata*, *P. umbilicalis*, *P. palmata* and *A. esculenta* were supplied by Wild Irish Seaweed Ltd. (Quilty, Co. Clare, Ireland). *U. pinnatifida* was bought from Algamar (Pazos de Borbén, Pontevedra, Spain) and *L. japonica* fucoxanthin powder extract was shipped by Nutra Green Biotechnology Co. Ltd. (Shanghai, China). Seaweeds were harvested along the west coast of Ireland, the north coast of Spain or in China. The seaweeds from Wild Irish Seaweed were air dried and dehumidified (Caherush Point, Spanish Point, Co. Clare, Ireland) and Algamar seaweeds were dried at low temperature (<42 °C) at Polígono de Amoedo (Pazos de Borbén, Pontevedra, Spain) to preserve them (Rodrigues et al., 2015). The fucoxanthin powder extract was produced by water and ethanol extraction. These seaweed species were chosen on the basis of abundance in Western European waters and potential suitability as ingredients in the formulation of new products. The fucoxanthin extract was included for comparative purposes.

3.3.2. Sample preparation

Dried seaweeds were milled to a particle size of 1 mm by means of a mechanical grinder. Milled samples were stored vacuum packaged in a -20 °C freezer for no longer than 30 days.

3.3.3. Compositional analysis

The moisture content of 2 g of seaweed was determined (Table 3.1) by drying the sample in a preheated (135 °C) oven for 2 h (Association of Official Analysis Chemists International (AOAC), 1996). The fat content of seaweed (5 g) was

determined using Foss Soxtec Avanti 2055 Manual system (Foss, Hillerød, Denmark). Seaweed samples were inserted into 33 mm x 80 mm extraction thimbles and extracted with 80 ml of boiling chloroform: methanol (2:1) for 30 min. Subsequently, thimbles were presented in the rinsing position for an additional 38 min. The extraction cups were removed and solvent was evaporated at 103 °C in an oven until a constant weight was obtained. The fat content was determined gravimetrically.

The protein content of 0.5 g of seaweeds was measured (Table 3.1) using the Kjeldahl method (Suhre et al., 1982). On completion, the content of the receiver flask was titrated with 0.1 M hydrochloric acid until the green colour reverted back to the original red colour. Finally, the protein was calculated using a nitrogen factor of 6.25. All analysis ware performed in duplicate.

The ash content of seaweed was determined (Table 3.1) in duplicate by a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) (Association of Official Analytical Chemists, 1923), which was pre-heated to 600 °C. Approximately 5 g of blended sample was weighed into porcelain dishes and placed into the muffle furnace. Samples were heated for 2 h until a white ash was obtained, at which time they were put in a desiccator to cool down. The ash content was calculated by weight of the dishes before and after sample introduction.

The salt content of seaweed was ascertained (Table 3.1), in duplicate, by titration using silver nitrate (0.1 M). Silver nitrate solution was standardised against 0.100 % sodium chloride solution. Two grams of sample were inserted into the muffle furnace to create ash for ash content analysis. Ash was washed into conical flasks with 20 ml distilled water and 2 ml of added chromate indicator. The flasks were potentiometrically titrated with 0.1 N silver nitrate from a clear yellow to an opaque light orange (+255 mV) after cooling to room temperature. Blank titration was performed using 20 ml distilled water.

Table 3.1. Composition of five seaweeds and a seaweed extract.

	HE	**UP**	**PU**	**PP**	**AE**[†]	**LJ**	
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	*P*-value
% Moisture	16.31 ± 0.43^{a}	10.77 ± 0.07^{b}	8.99 ± 0.22^{c}	10.04 ± 0.38^{b}	11.79 ± 0.12^{d}	6.68 ± 0.6^{e}	<0.001
% Fat[††]	1.42 ± 0.17^{a}	2.30 ± 0.12^{b}	1.13 ± 0.03^{a}	1.69 ± 0.17^{a}	2.30 ± 0.07^{c}	0.38 ± 0.03^{d}	<0.001
% Protein[††]	5.84 ± 0.44^{a}	9.02 ± 0.55^{b}	33.57 ± 1.4^{c}	19.96 ± 1.11^{d}	8.82 ± 0.31^{e}	0.6 ± 0.14^{f}	<0.001
% Ash	35.92 ± 0.45^{a}	57.36 ± 0.35^{b}	18.94 ± 0.31^{c}	28.49 ± 0.75^{d}	35.03 ± 0.49^{e}	5.93 ± 0.29^{f}	<0.001
% Salt	15.16 ± 0.25^{a}	36.77 ± 1.93^{b}	5.88 ± 0.13^{c}	16.34 ± 1.06^{a}	17.31 ± 0.21^{d}	-	<0.001

HE, *Himanthalia elongata*; UP, *Undaria pinnatifida*; PU, *Porphyra umbilicalis*; PP, *Palmaria palmata*; AE, *Alaria esculenta*; *Laminaria japonica* (fucoxanthin extract); M, mean; SD, standard deviation. $^{a\text{-}f}$, mean values in the same row bearing different superscripts indicate significant difference (P <0.05) between the seaweed species. [†]Mohammed HO et al. Characterization of the nutritional and bioactive properties of brown and red Irish seaweeds for potential use as functional ingredients in processed meat products. *47th Annual Food Science and Technology Conference* Cork, Ireland; 2018. [††] Percentage on dry weight basis.

3.3.4. Extraction and volatile compounds analysis

3.3.4.1. Thermal desorption

The extraction of 10 g of dried milled seaweed by TD GC-MS was performed as previously described in Garicano Vilar et al. (2020) (Garicano-Vilar et al., 2020a) for 40 min at 72 °C using a micro-chamber/thermal extractor (Markes International Ltd., Llantrisant, UK). The set of check standards used were 1-butanol, dimethyl

disulphide, butyl acetate, cyclohexanone, benzaldehyde and 2-phenyl-D5-ethanol, at 0.5 g Kg^{-1}.

3.3.4.2. Headspace solid-phase microextraction

HS-SPME analysis was performed as described by Lamichhane et al. (2018) (Lamichhane et al., 2018), except that 3 g of dried milled seaweed (or powder extract) was extracted for 20 min at 40 °C, and the mass range scanned was 35-350 amu. Batch processing of samples was carried out using MetaMS, an open-source pipeline for GC-MS-based untargeted metabolomics (Wehrens et al., 2014a). A multi-phase SPME 50/30 µm divinylbenzene/carboxen™/polydimethylsiloxane (DVB/CAR/PDMS) (Agilent Technologies Ltd., Cork, Ireland) was used because the properties of the different phases are utilized to target chemical classes based on their polarity and volatility and molecular weight. Previous studies have shown that fiber choice was suitable for the extraction of volatile compounds in seaweeds (Balbas et al., 2015; López-Pérez et al., 2017).

3.3.5. Gas chromatography-Olfactometry analysis for aroma-active compounds

The extraction of volatile compounds was carried out by HS-SPME using a Gerstel MPS autosampler (Anatune Ltd., Cambridge, UK). The same SPME fibre (DVB/CAR/PDMS) was used as described earlier. The fiber was pre-conditioned before use at 270 °C for 60 min. Seaweed samples (dried seaweed and extract) (3 g) were weighed in 20 ml amber SPME La-Pha-Pack headspace vials with magnetic caps and silicone/polytetrafluoroethylene (1.3 mm 45° Shore A) septa (Apex Scientific Ltd., Maynooth, Ireland). The SPME fibre was exposed to the seaweed headspace at 40 °C for 60 min at a depth of 1 cm. Injections were carried out in splitless mode, with the injector temperature at 250 °C. All samples were analysed in triplicate.

The GC-O analyses were conducted on an Agilent 7890A GC coupled with an Agilent 5975C mass selective detector (MSD) (Agilent Technologies Ltd., Cork,

Ireland). The GC was also equipped with a flame ionization detector (FID) and a Gerstel olfactory detection port ODP3 with heated mixing chamber (Anatune Ltd., Cambridge, UK). A humidifying device was used to reduce nasal mucosa dehydration. Analyses were performed on a DB-624 column (20 m x 1.80 mm i.d. x 1 µm phase thickness). Helium was used as a carrier gas at a flow rate of 1.2 ml/min and nitrogen as auxiliary gas. The GC oven temperature was programmed to increase from 60 to 180 °C at a rate of 6 °C/min, with an initial hold time of 2 min, and from 180 to 220 °C at a rate of 15 °C/min, with a final hold time of 5 min. The total run time was 29.66 min. Column effluent was split equally between the FID, the olfactory port and the MSD. The transfer line into the MSD was kept at 260 °C. The FID temperature was set at 300 °C, with an air flow of 400 ml/min, a H_2 fuel flow of 30 ml/min and a N_2 makeup flow of 25 ml/min. The sniffing port and its exit were maintained at 150 °C and 40 °C, respectively.

A panel of three sensorial assessors (age 26-39 years) carried out the sniffing of the volatile compounds of seaweeds extracted by SPME. Sniffing time was approximately 30 min and each assessor carried out one session per day. The panellists were asked to rate (i) the intensity of the eluted aroma using a four-point category scale (1 = weak, hardly recognizable odour; 2 = clear but not intense odour, 3 = intense odour, 4 = very intense odour), recorded by a Gerstel OID Interface/ODP-Recorder (Anatune Ltd., Cambridge, UK) and (ii) the odour perceived, by voice recording. The odorants taken as significant were the ones in which at least two assessors were able to detect. None of the assessors was anosmic, as tested by the Sniffin' Sticks test (Burghardt Messtechnik, Wedel, Germany) prior to the GC-O analysis (Rumeau et al., 2016).

Tentative identifications were based on comparison of the mass spectra of unknown compounds against those of the National Institute of Standards and Technology (NIST), or by comparing the retention index value to values available in the literature and an in-house library. The odorants were also identified by comparison of their odours with Flavornet database [http://www.flavornet.org], The Good Scents Company database [http://www.thegoodscentscompany.com] and other specified publications. Retention indices for all detected volatile compounds

were calculated using an n-alkane series (Merck, Arklow, Ireland) for both GC-MS and GC-O analysis.

3.3.6. Descriptive odour evaluation

Three panellists attended a focus group session on descriptive terms prior to the sensory analysis. The six samples were evaluated in one session, following the ISO 6658:2005 recommendation (International Organization for Standardization, 2005). All samples were assessed only for odour attributes and analysed in duplicate by each panellist. Mean scores for each attribute were calculated. The assessors quantified the attributes using a ten-point scale anchored to the left with 'not' and to the right with 'very' (Meilgaard et al., 2006). Two grams of each sample was put in 20 ml amber glass vials with screw caps and the vials were kept in a HiSorb Agitator (Markes International Ltd., Llantrisant, UK) at 40 °C for 20 min at a rotational speed of 350 rpm (mn^{-1}), to ensure the accumulation of volatiles in the headspace. Samples were provided randomly to each panellist. The testing area used in this study complied with the ISO 8589:2007 standards (International Organization for Standardization, 2007b).

3.3.7. Data analysis

Normality and homogeneity were examined and a one-way analysis of variance (ANOVA) with the Bonferroni test for *post hoc* analyses was carried out to assess statistical differences between seaweed species in terms of composition and abundance values of volatile compounds, at a significance level of $P = 0.05$, using the statistical package for the social sciences (SPSS) software version 24.0 (IBM Corp., Armonk, NY, USA).

The volatile profile was analysed using R software (RStudio, Inc., Boston, MA, USA) for statistical computing and graphics. Principal component analysis (PCA) was carried out to visualize the differences in volatile aroma composition detected by GC-

MS, GC-O analysis and sensory panel results, and to elicit the main factors contributing to the differences between the six samples.

In addition, volatile compounds and odour intensity values were further analysed using ANOVA-partial least squares regression (APLSR) with Unscrambler X Software, version 10.3 (CAMO ASA, Trondheim, Norway) (see Fig. S-3.1).

3.4. Results and discussion

3.4.1. Physiochemical analysis

The composition of the five seaweeds and the extract are summarized in Table 3.1. The moisture content was statistically different (P <0.01) among all species, with the exception of *U. pinnatifida* and *P. palmata*. The data resemble the proximate composition of three brown seaweeds analyzed by Lorenzo et al. (Lorenzo et al., 2017), who reported that moisture content ranged from 7.95 % in *B. bifurcata* to 11.2 % in *A. nodosum* and *F. vesiculosus*. Rodrigues et al. (Rodrigues et al., 2015) also reported similar moisture contents: 9.6-10.9 % in brown seaweeds and 8.0-11.8 % in red seaweeds.

According to Ibañez et al. (Ibañez and Cifuentes, 2013), brown seaweed species (Phaeophyceae) are characterized by a lower protein content in comparison to red seaweed species (Rhodophyta). In effect, the red seaweeds (*P. umbilicalis* and *P. palmata*) presented a higher proportion of protein than the brown seaweeds (Table 3.1). The protein content was statistically different (P <0.001) among all species tested. These findings are similar those of Lorenzo et al. (Lorenzo et al., 2017), where the protein content of brown species ranged from 8.7 to 13.0 %. However, Rodrigues et al. (Rodrigues et al., 2015) reported higher (14.4 to 16.9 %) protein content for brown species and lower protein content (20.2 to 23.8 %) for red species.

Seaweeds are well known for their low fat content, but values vary considerably between studies. A range of 1.1 and 2.3 % of total fat was found in our samples (Table 3.1). The fat content of red seaweed did not have any significant differences, but significant differences were evident for green seaweed (P <0.01).

Higher fat content of 0.9 and 0.6 % were reported in red species of *O. pinnatifida* and *Gracilacia gracilis*, respectively, by Rodrigues et al. (Rodrigues et al., 2015). Higher fat content was also observed in brown species in this study compared to those reported by Jard et al. (Jard et al., 2013) for *Sargasum muticum*.

Higher levels of ash are associated with higher amounts of minerals (Ismail, 2017). The ash content of *P. umbilicalis* and *P. palmata* was lower than that of brown seaweed samples. *Undaria pinnatifida* had the highest ash content (Table 3.1). Greater variability in total ash content was observed in brown seaweed than in red seaweed. The differences between the seaweed species were all statistically significant (P <0.001), even between red and brown seaweeds. The ash content values observed in this study are in agreement with published values for *Grateloupia turuturu* (18.5 %) by Denis et al. (Denis et al., 2010) and for *G. gracilis* (24.8 %) by Rodrigues et al. (Rodrigues et al., 2015). In contrast, higher values of ash content were observed in brown species in this study than in other studies (Lorenzo et al., 2017; Rodrigues et al., 2015). The salt content of all seaweeds followed the same trend as the ash content (Table 3.1), although no statistically significant difference was observed between *H. elongata* and *P. palmata*.

3.4.2. Volatile compounds identification

A total of 117 (Table S-3.1) and 109 (Table S-3.2) volatile compounds were detected in the five seaweed species and in the seaweed extract by TD and HS-SPME, respectively. These numbers were lower than the 151 volatile compounds identified by López-Pérez et al. (López-Pérez et al., 2017) in seven species of dehydrated edible seaweeds. The number of volatile compounds identified in each individual seaweed species by TD and HS-SPME, respectively, were 75 and 61 compounds for *H. elongata*, 76 and 58 for *U. pinnatifida*, 65 and 58 for *P. umbilicalis*, 78 and 76 for *P. palmata*, 72 and 60 for *A. esculenta,* and 67 and 53 for the fucoxanthin extract. These numbers were lower than the 129, 140, 131 and 136 volatile compounds found by López-Pérez et al. (López-Pérez et al., 2017) in *H. elongata*, *U. pinnatifida*, *P. umbilicalis* and *P. palmata*, respectively; but higher than the 23 volatiles in *P. palmata* samples (Le Pape

et al., 2004) or the 26 and 24 volatile compounds in *Laminaria* spp and *U. pinnatifida*, respectively (Ferraces Casais et al., 2013). The differences may be due to a myriad of reasons; species, geographical area of growth, harvesting, storage and volatile extraction conditions amongst others. As previously mentioned, no single extraction technique can provide a complete volatile profile as they have an inherent bias due to the different adsorbent and absorbent properties of the different phases, molecular sieve characteristics, mechanisms of extraction and the physical parameters used. In this study we carried out HS-SPME at 40 °C and extracted for 20 min, and we carried out TD at 72 °C for 40 min in an attempt to extract more compounds as much more sorbent capacity exists for TD than HS-SPME and larger sample amounts of sample were used (10 g versus 3 g) (Fig. S-3.2 and S-3.3). As mentioned, the properties of the sorbent materials used in these techniques are different and will therefore extract and concentrate different VOC. The TD tubes contained Tenax Carbograph, which is useful for the detection of VOC between C3 and C30 of different volatilities (typically 50-450 °C) and polarity. Limitations occur for some low-molecular-weight compounds lower than C3, especially if they are polar, such as carboxylic acids, some aldehydes, and alcohols (Schieweck et al., 2018). The SPME fiber (DVB/CAR/PDMS) is useful for a wide range of volatiles and semi-volatiles between C3-C20. This fiber affords a greater extraction efficiency for many VOC, in comparison to other fibers (PDMS-DVB, CAR-PDMS, PDMS or polyacrylate) and is particularly useful for very volatile VOC but less useful for heavier VOC such as lactones (Ducki et al., 2008; Garcia-Esteban et al., 2004; Mondello et al., 2005). It is therefore useful to utilize different VOC extraction / concentration techniques to achieve a more representative VOC profile.

From the TD analysis the highest numbers of volatile compounds were, in decreasing order; 23 alcohols, 20 ketones, 20 aldehydes, 9 acids, 8 esters, 7 furans, 6 benzenes, 5 pyrazines, 5 terpenes, 4 sulphur compounds, 3 ethers, 3 lactones, 2 terpenes, 2 pyridines, 1 phenol and 1 chlorine; with HS-SPME detecting 21 ketones, 20 aldehydes, 16 alcohols, 9 acids, 9 benzenes, 7 esters, 6 furans, 6 pyrazines, 5 terpenes, 3 sulphur compounds, 3 lactones, 2 ethers, 1 phenol and 1 chlorine. The different volatiles detected by both extraction techniques highlight the merits of using more than one extraction technique to encompass a larger volatile profile.

Overall, terpenes were the most prominent in *H. elongata*; acids and lactones in *U. pinnatifida*; sulphur compounds in *P. umbilicalis* (HS-SPME extraction only); aldehydes, esters, ketones, pyrazines and pyridines in *P. palmata*; alcohols, ethers and furans in *A. esculenta*, and benzenes and phenol were predominant in the fucoxanthin extract.

3.4.3. Descriptive odour analysis

Descriptive odour analysis was conducted on the five different species of seaweed and the seaweed extract (Fig. 3.1). The samples were classified and characterized by 'rancid, oily/fatty, pungent', 'fruity, citrus', 'grassy, herbal, floral, vegetable', 'fresh, marine, fishy', 'nutty, toasted', 'mushroom, earthy, damp' and 'sweet, buttery, creamy' aroma. A PCA was produced to visualize the differences in the aroma attributes between the species and the extract (Fig. 3.1). The first two principal components of the PCA were able to explain 81 % of the variance (Fig. 3.1). Globally, *H. elongata* and *P. palmata* were mostly characterized by 'fresh, marine, fishy' aroma; *U.pinnatifida* and *A. esculenta* by 'grassy, herbal, floral, vegetables' notes; *P. umbilicalis* by 'mushroom, earthy, damp' and 'rancid, oily, fatty' notes and fucoxanthin extract by 'nutty, toasted' aroma (Fig. 3.1 and Fig. 3.2).

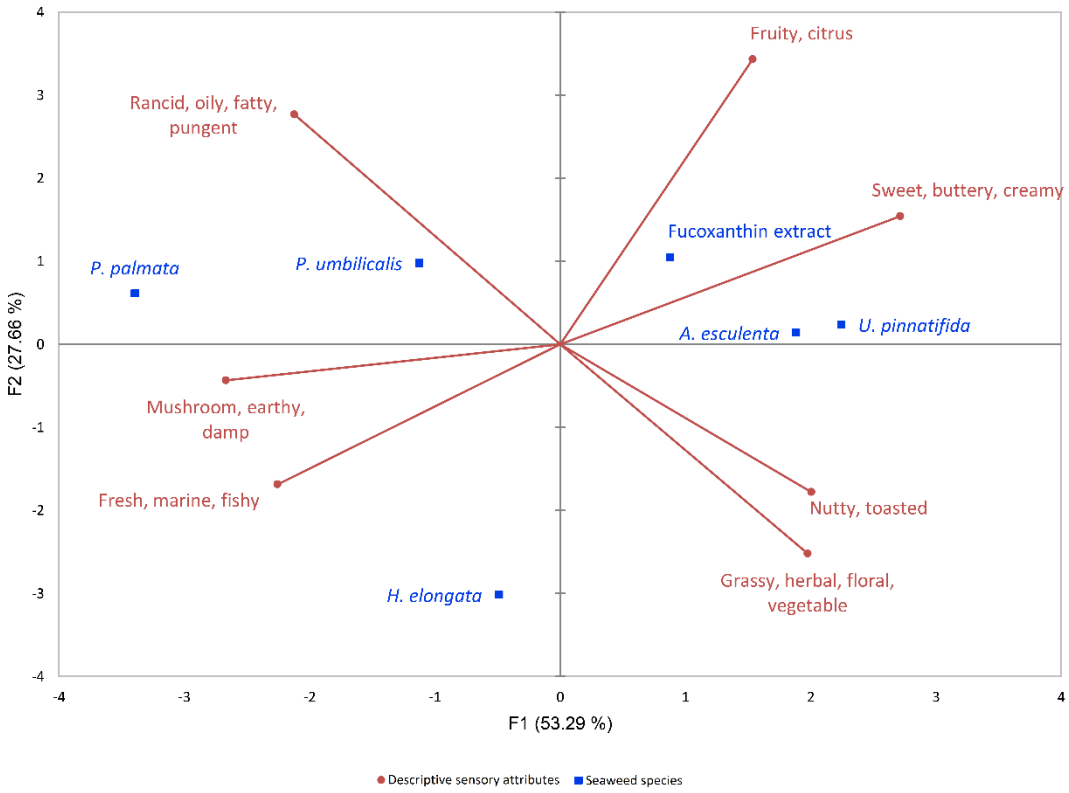


Figure 3.1. Principal component analysis (PCA) scores plot of aroma evaluation of five seaweed samples (*H. elongata*, *U. pinnatifida*, *P. umbilicalis*, *P. palmata*, and *A. esculenta*) and one seaweed extract (fucoxanthin extract from *L. japonica*), for the first two principal components (PC1 and PC2). Average scores are according to quantitative descriptive odour attributes evaluated by three panellists.

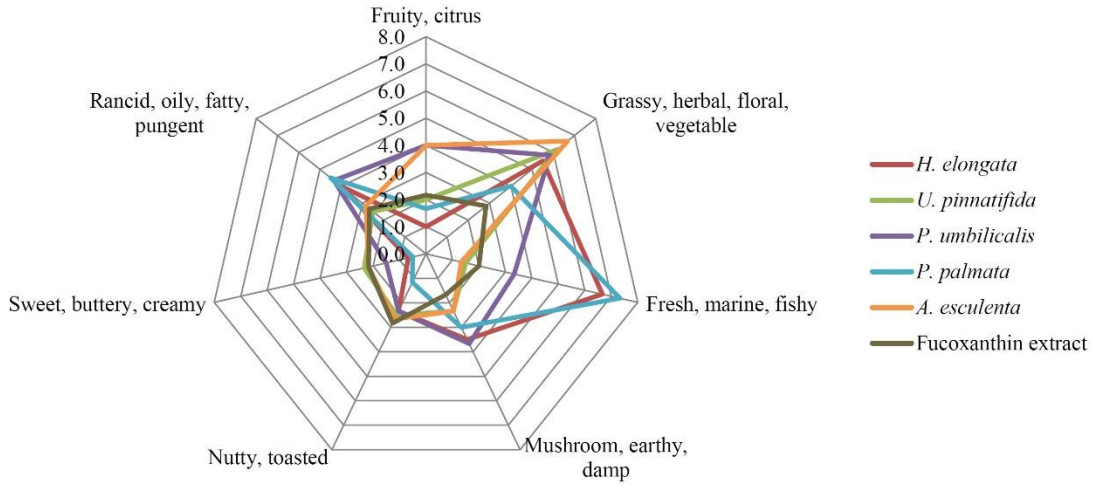


Figure 3.2. Spider diagram of the sensory evaluation of five seaweeds (*H. elongata*, *U. pinnatifida*, *P. umbilicalis*, *P. palmata*, and *A. esculenta*) and one seaweed extract (fucoxanthin extract from *L. japonica*). Average scores are shown according to quantitative descriptive odour attributes evaluated by three panellists.

3.4.4. Odour characteristics of aroma-active compounds determined by HS-SPME-GC-O

In total 41 aroma active volatiles were identified, and 5 unidentified aroma-active compounds were perceived by panellists, consisting of acids, alcohols, aldehydes, ethers, furans, ketones and pyrazines (Table 3.2). If a volatile compound is present at a concentration above its odour threshold but below its limit of detection it cannot be identified but its aroma will be perceived by GC-O. Quite a few of the compounds were also summarized as having odour descriptors different or partially different from the referenced descriptors. However, this is also not unusual as odour descriptors can be influenced by the concentration (odour descriptors can change based on concentration for some volatile compounds), the matrix effect (the composition of the sample can influence the release of volatiles into the headspace) and differences in the ability of the human assessors due to genetic and cultural differences (Ferdenzi et al., 2017).

Table 3.2. Aroma-active compounds detected in five species of edible seaweeds and a seaweed extract by GC-O.

Compound	RI[†]	LRI[‡]	IM[§]	Odour[¶]	Odour intensity[††]						Odour ref. [a-i]	Odour threshold (ppb) [j-n]
					HE	UP	PU	PP	AE	LJ		
ACIDS												
Acetic acid	687	690	A, C	Vinegar	1.6	2.6	-	-	-	-	Vinegar, sharp, sour [a]	480-1000 [j]
Propanoic acid	780	784	A, C	Cheese, rancid, medical	-	2	-	-	-	-	Pungent, acidic, cheesy, vinegar, dairy [a]	20000 [k]
ALCOHOLS												
Ethanol	483	506	A, C	Alcohol	-	1.3	-	-	0.6	-	Strong, alcoholic, ethereal, medical [a]	100000 [k]
1-Propanol	610	615	A, C	Sweet	-	-	1.3	-	-	-	Alcoholic, fermented, tequila, musty, yeasty, sweet, fruity, apple, pear [a]	9000 [k]
2-Furanmethanol	931	939	B, C	Cheese, butter, buttermilk, rancid	-	-	-	-	-	2.6	Alcoholic, chemical, musty, sweet, caramel, bread, coffee [a]	8000 [l]
2-Penten-1-ol, (Z)-	825		B, C	Sweet, cherry, fruity, candy	3	-	-	-	-	-	Cherry, narcissus, fruity, green, phenolic, nasturtium, ethereal, medicinal, aldehydic, metallic [a]	Nf

1-Pentanol	817	815	A, C	Cotton candy, fruity	-	-	1.6	-	-	-	Pungent, fermented, bready, yeasty, fusel, winey, solvent [a]	4000 [k]
1-Hexanol	919	916	B, C	Lemon, citrus, grass	-	-	-	-	1	-	Fruity, alcoholic, sweet, green, pungent, ethereal, fusel, oily [a]	2500 [k]
1-Octen-3-ol	1028	1030	A, C	Mushroom, mineral, hay	2	2.3	2	2	3.3	-	Mushroom, earthy, green, oily, fungal [a-c]	1 [k]
ALDEHYDES												
Propanal, 2-methyl-	598	592	A, C	Vanilla, floral	-	-	1	1.3	-	-	Fresh, aldehydic, floral, pungent [a]	0.1-2.3 [k]
Pentanal	733	735	A, C	Caramel, sugar, spicy, butter	-	-	1.6	-	-	-	Fermented, bready, fruity, nutty, berry [a]	12-42 [k]
Butanal, 3-methyl-	694	692	A, C	Vanilla, sweet, floral, butter	-	-	1.3	2.3	-	-	Ethereal, aldehydic, chocolate, peach, fatty [a]	12 [m]
Hexanal	837	839	A, C	Herbal, grassy	2.3	-	1.6	1.6	-	-	Fresh, green, grassy, leafy, fruity, sweaty, woody, clean [a,c]	5 [k]
2-Pentenal, 2-methyl-	877		B, C	Sweaty, milk, rancid	-	-	-	2	-	-	Pungent, fruity, juicy, ripe, aldehydic, green, grassy, gassy [a]	290 [n]
Benzaldehyde	1034	1030	B, C	Green, grass, vegetable, herbal, almond	-	-	-	1.3	3	-	Almond, burnt sugar-like, cherry, bitter, sharp, fruity, sweet, bitter almonds [a,c-e]	350-3500 [k]

2,4-Heptadienal, (E,E)-	1074		B, C	Grassy, vegetable, cucumber	-	-	-	2.3	-	-	Fatty, green, oily, aldehydic, vegetable, cinnamon [a]	3600 [m]
Heptanal	942	943	A, C	Fatty, oily, fishy, seaweed, lake water	3.3	3.6	4	3.6	3.3	2	Fresh, aldehydic, fatty, green, herbal, wine-lee, ozone, citrus, rancid [a,c,f]	3-60 [n]
Benzeneacetaldehyde	1109	1119	A, C	Tea, floral	-	0.6	-	-	-	-	Harsh, green, honey, cocoa, sweet, floral, hyacinth, clover, rose, powdery, fermented [a,f,g]	4 [k]
2-Octenal, (E)-	1116		B, C	Green, grass, pepper, coffee	-	-	-	1.6	-	-	Fresh, cucumber, fatty, green, herbal, banana, waxy, leafy, nut [a,f]	3 [k]
2,6-Nonadienal, (E,Z)-	1216		B, C	Fresh, cucumber, grass, green	3.3	3	-	3	-	-	Green, cucumber, melon, fatty, vegetable, dry, violet, leaf [a,c]	0.01 [k]
2-Nonenal, (E)-	1218		B, C	Fresh, lemon, grassy, nuts	2.3	-	-	-	-	-	Green, cucumber, aldehydic, fatty, citrus [a]	0.08-0.1 [k]
ETHERS												
Butanoic acid, ethyl ester	747	828	B, C	Fruity, cherry, strawberry, candy	-	2.6	2	2.3	-	-	Sweet, fruity, tutti frutti, juicy [a]	1 [k]
Octanoic acid, methyl ester	1151	1156	B, C	Fermented, fresh, green	-	-	1	-	-	-	Waxy, green, sweet, orange, aldehydic, vegetable, herbal [a]	200 [k]
FURANS												

Furan, 2-pentyl-	1009	1012	A, C	Fried, melted butter, toasted, herbal	2	-	-	1.3	-	-	Fruity, green, earthy, beany, vegetable [a,c]	6 [k]
Furan, 2-hexyl-	1386		B, C	Fruity, floral, green, tea	1.6	-	-	-	-	-	Floral, pulpy [h]	Nf
KETONES												
Acetone	565	533	A, C	Boiled corn, rocket	-	-	1	-	-	-	Solvent, etheral, apple, pear [a]	500000 [k]
2,3-Butanedione	627	631	A, C	Butterscotch, sweet, yeast, butter, vanilla, custard	-	2.6	1	-	1.3	1	Buttery, sweet, creamy, caramellic, pungent [a,c,f]	2.3-6.5 [k]
2-Butanone	636	639	A, C	Butterscotch, sweet, vanilla, cream, fruity	1.3	-	-	2.3	-	-	Camporheous, fruity, ethereal, acetone, nauseating odor [a,c,e]	50000 [k]
2-Heptanone	934	928	A, C	Plastic, grassy	-	-	-	1.3	1.6	-	Fruity, spicy, sweet, herbal, coconut, woody, sulfur, pungent, green [a,f]	14-3000 [k]
3-Heptanone	927		A, C	Fruity, rancid, butter, oily	2	2.6	-	3.3	3.3	-	Green, fatty, fruity [a]	Nf
3,5-Octadien-2-one	1131		B, C	Metallic, mineral	1.6	0.6	-	2	-	-	Fruity, fatty, mushroom [a]	0.15 [n]
Sulcatone	1032		A, C	Woody, green, grass, celery, vegetable	3.3	3	-	3	-	-	Citrus, green, musty, lemongrass, apple, cheesy, banana [a]	50 [k]

Isophorone	1108		B, C	Grapefruit, spicy, wet, rosy, floral, caramel, sugar	-	-	-	2	1.3	-	Cooling, woody, sweet, green, camphor, fruity, musty, cedar, wood, tobacco, leather [a]	200 [l] (air)
PYRAZINES												
Pyrazine, 2,6-dimethyl-	947	944	A, C	Backed, toasted, roasty, caramel	-	-	-	3.6	-	-	Cocoa, roasted, nutty, meaty, coffee [a]	400-1500 [n]
TERPENES												
Dihydroactinidiolide	1701		B, C	Damp, floral	-	-	-	1.6	-	-	Ripe, apricot, fruity, berry, woody [a]	Nf
α-Ionone	1500		B, C	Grassy, lemon, citrus, sugary, fruity	-	-	-	1.6	-	-	Sweet, woody, floral, violet, orris, tropical, fruity [a]	0.6-10 [n]
OTHERS												
Safranal	1260		B, C	Grassy, vegetable, anise, fennel	1.3	-	-	1.6	1	-	Fresh, herbal, phenolic, metallic, rosemary, tobacco, spicy, woody, camphoreous, medicinal, powdery, herbal [a]	Nf
Nonane, 3-methylene-	991		B, C	Incense, spices, earthy, floral, sweet, cherry, strawberry	-	-	1.3	2.3	-	-	Nf	Nf
Nonane, 2-methyl-	965		B, C	Baked, toasted	-	-	-	-	-	1.3	Nf	Nf
1-decene, 2,4-dimethyl-	1229		B, C	Grass, green	-	-	2.3	-	-	-	Nf	Nf
Dodecane	1199		B, C	Soap, glue	2	1.3	-	1.3	-	-	Gasoline-like [i]	Nf

Unidentified-1	961	C	Toasted, yeast, fermented, roasted, nutty	-	-	3	-	-	-	
Unidentified-2	969	C	Pyrazine, grass, boiled potato	-	-	3	3.6	-	-	
Unidentified-3	1032	C	Floral, geranium, green, grass, damp, mouldy	-	-	3	-	-	-	
Unidentified-4	1143	C	Burning wood, hay, grass	-	-	-	-	1.6	-	
Unidentified-5	1226	C	Rubber, plastic	-	-	-	-	-	1.6	
			Total odour intensity	32.9	28.1	32	54.1	21.3	8.5	

HE, *Himanthalia elongata*; UP, *Undaria pinnatifida*; PU, *Porphyra umbilicalis*; PP, *Palmaria palmata*; AE, *Alaria esculenta*; LJ, *Laminaria japonica* (fucoxanthin extract); [†] Retention index (RI) calculated from GC-O results on a DB-624 UI column; [‡] Retention index found in the literature (LRI) for a DB-624 UI column; [§] Identification method (IM): A, identification based on NIST mass spectral database, RI values from the literature and an in-house library created using authentic compounds with target and qualifier ions and linear RI for each compound; B, when only B or RI values were available, it must be considered as a tentative identification; C, identification with GC-O; [¶] Odour description given by three assessors during GC-O analysis. -, not detected; [††] Mean odour intensity for each seaweed species evaluated by sniffers. From 1 = low to 4 = high; [a-i] Odour descriptors found in the literature: **a** [http://www.thegoodscentscompany.com/index.html], **b** [Isleten Hosoglu M. *Food Chem* 240:1210–1218 (2018)], **c** [Narain N. *Examines Mar Biol Oceanogr* 2:195–201 (2018)], **d** [Minteguiaga M et al. *J Sep Sci* 38:3038–3046 (2015)], **e** [Flament I. 1st ed. John Wiley & Sons Ltd., ed. Chichester, England; 2002], **f** [Dong L et al. *J Sci Food Agric* 95:915–921 (2015)], **g** [Lasekan O et al. *CYTA - J Food* 14:154–161 (2015)], **h** [de

Sousa Galvão M et al. *Food Res Int* 44:1919–1926 (2011)], **i** [Verma DK et al. 1st ed. Verma DK, Srivastav PP, eds. Sci. Technol. Aroma, Flavor, Fragr. Rice. Oakville, Canada: Academic Press, Inc; 2019]. Nf, not found; [j-n] Odour threshold values in water (unless otherwise designated) found in the literature: **j** [New Jersey Department of Health. Hazardous Substance Fact Sheet Acetic Acid. 2016], **k** [http://www.leffingwell.com/odorthre.htm], **l** [U.S. National Library of Medicine and National Center for Biotechnology Information, 2016], **m** [American Society for Testing and Materials. 2nd ed. Fazzalari FA, ed. Baltimore, Md.: American Society for Testing and Materials; 1978], **n** [Burdock GA. *Fenaroli's Handbook of Flavor Ingredients, Fourth Edition*, 2001]. Nf, not found.

On the one hand, *P. palmata* had the strongest odour of all samples, as the sum of olfactory scores was the highest at 54.1 (Berglund et al., 1973) (Table 3.2). On the other hand, fucoxanthin extract had the lowest score (8.5), resulting in a rather bland aroma profile that was dominated by 'backed, buttery, sweet, fatty' aromas. This is possibly due to the additional processing required to produce the extract. In addition to heptanal, and taking into account the olfactory scores for each compound individually, (E,Z)-2,6-nonadienal (fresh, cucumber, grass, green) and sulcatone (woody, green, grass, celery, vegetable) appear to be important aroma compounds for *H. elongata* and *U. pinnatifida*. *Porphyra umbilicalis* was characterized by 3 unidentified compounds with (i) 'toasted, yeast, fermented, roasted, nutty', (ii) 'pyrazine, grass, boiled potato' and (iii) 'floral, geranium, green, grass, damp, mouldy' aromas. *Palmaria palmata* was dominated by 2,6-dimethyl-pyrazine (baked, toasted, roasty, caramel), an unidentified component with 'pyrazine, grass, boiled potato' aroma and 3-heptanone (fruity, butter, oily). *Alaria esculenta* was characterized by 1-octen-3-ol (mushroom, earthy), 3-heptanone (fruity, rancid, butter, oily) and benzaldehyde (green, grass, vegetable, herbal, almond). 2-furanmethanol (cheese, butter, buttermilk, rancid) and 2-methyl-nonane (backed, toasted) appeared to be characteristic aroma notes for only the fucoxanthin extract but at low (<3) intensities.

As mentioned, GC-O analysis showed that 1-octen-3-ol (mushroom, mineral, hay) and heptanal (herbal, grassy) were common in all samples but differed in their intensities. The compound 1-octen-3-ol contributes to 'mushroom' and 'mushroom-metallic' notes (Isleten Hosoglu, 2018; Van Durme et al., 2013b). Moreover, 1-octen-3-ol has been shown to contribute to the formation of seafood aroma (Van Durme et al., 2013b). Heptanal contributes to 'green' and 'fresh' aroma (Narain, 2018). It has also been described as having 'fat', 'rancid', 'citrus' aromas (Dong et al., 2015). Heptanal, which was the potent aroma compound for all samples in this study, exhibited a 'fatty, oily, fishy, seaweed, lake water' aroma and appears to contribute to the characterizing notes of the fish, seaweed, oil aromas of seaweed. Many short chain aldehydes (e.g., 2-octenal) can also provide several aromas to food matrices (fatty, green, woody, fatty, nutty, floral, citrus, waxy, and sweet) depending on the number of carbon atoms and the degree of saturation (Van Durme et al., 2013b).

Many volatile compounds in a food present low aroma intensity or no aroma and thus do not necessarily contribute to the overall odour (Kamadia et al., 2006). Thus, high threshold compounds can present low odorant power, even if present at a high abundance. Conversely, low concentrations of low threshold compounds in the sample can present a high odour intensity (da Rocha et al., 2017). It is therefore necessary to know if the extracted volatile compounds are actually contributing to the characteristic aroma.

A second PCA was performed on the seaweeds samples in order to determine the relationships between the volatile aroma active compounds as determined by GC-O analysis in the different seaweed species (Fig. 3.3). The first two principal components explain 59 % of the variance.

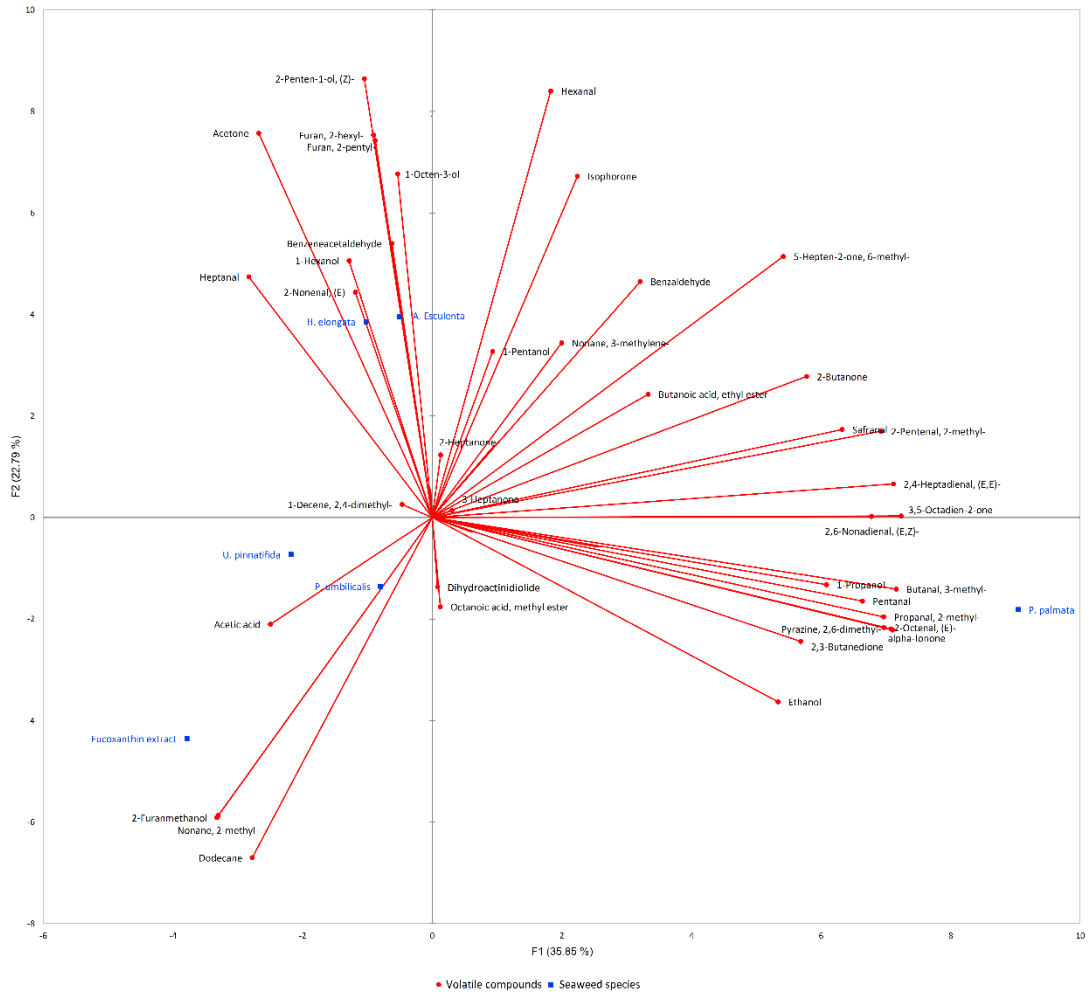


Figure 3.3. Principal component analysis (PCA) scores plot depicting the distribution of aroma compounds detected by GC-O analysis in five seaweed species (*H. elongata*, *U. pinnatifida*, *P. umbilicalis*, *P. palmata*, and *A. esculenta*) and one seaweed extract (fucoxanthin extract from *L. japonica*), for the first two principal components (PC1 and PC2).

A clear discrimination exists between these samples based on their olfactometry assessment (Fig. 3.3). Both *H. elongata* and *A. esculenta* are quite similar, on the positive side of F2 and the negative side of F1. These samples are associated with heptanal (fatty, oily, fishy, seaweed, lake water), (E)-2-nonenal (fresh, lemon, grassy, nuts), 1-hexanol (lemon, citrus, grass), benzeneacetaldehyde (tea, floral), 1-octen-3-ol (mushroom, mineral, hay), 2-hexyl furan (fruity, floral, green, tea), 2-pentyl furan (butter, toasted, herbal), acetone (sulphur, boiled corn, rocket), (Z)-2-penten-1-ol (sweet, cherry, fruity, candy), 1-pentanol (cotton candy, fruity) and 2-heptanone (grassy, plastic). Only some of the individual odour descriptors match the overall aroma characteristic by hedonic assessment; however, during aroma evaluation the panellists are sniffing a combination of all these odours rather than individual odours by GC-O, thus combined odours may be perceived very differently (Thomas-Danguin et al., 2014). It is worth noting that odour thresholds and abundances are the main factors that impact on odour perception. It seems plausible that heptanal must be a key odorant in these species as it has an odour similar to their aroma attributes. Heptanal also has a mid- to low odour threshold, but a high odour intensity score (Table 3.2). The intensity scores for (E,Z)-2,6-nonadienal (Z)-2-penten-1-ol in *P. umbilicalis*, *U. pinnatifida* and the fucoxanthin extract were all on the negative side of F1 and F2 (Fig. 3.3). These were all associated with 'fruity, citrus', 'sweet, buttery, creamy' aroma attributes. Both *P. umbilicalis* and *U. pinnatifida* were strongly associated with acetic acid (vinegar), and *P. umilicalis* was also strongly associated with methyl octanoate (fermented, fresh, green). It appears that methyl octanoate contributes to the overall aroma character of *P. umilicalis* as its aroma is similar. The fucoxanthin extract was differentiated from all the other samples. It was associated more with 2-furanmethanol (cheese, butter, rancid), 2-methyl-nonane (toasted) and dodecane (soap, glue) and less with acetic

acid or methyl octanoate. The individual aromas of these volatiles, however, do not match the overall aroma characteristics. *Palmaria palmata* was also discriminated from all the other samples on Fig. 3.3, on the positive side of F1 and the negative side of F2. It was associated most with 'mushroom, earthy, damp' and 'rancid, oily, fatty, pungent' aroma attributes, but also with a very wide range of volatiles: 3-methyl-butanal (vanilla, sweet, floral, butter), pentanal (caramel, sugar, spicy, butter), 2-methyl propanal (vanilla, floral), 2,6-dimethyl pyrazine (backed, toasted, roasty, caramel), α-ionone (grassy, lemon, citrus, sugary, fruity), (E)-2-octanal (green, grass, pepper, coffee), 2,3-butanedione (butterscotch, sweet, yeast, butter, vanilla, custard), ethanol (alcoholic), 1-propanol (sweet), methyl octanoate (fermented, fresh, green), (E,Z)-2,6-nonadienal (fresh, cucumber, grass, green), 3,5-octadien-2-one (metallic, mineral) and dihydroactinidiolide (damp, floral). It appears that 3,5-octadien-2-one is likely a major contributor to the aroma of *P. palmata* due to its odour characteristics and intensity score (Table 3.2).

Panellists in the study by Peinado et al. (2014) used 'honey-like' odour, 'herbal' odour, 'seaweed-like' odour attributes to describe five different species of brown edible seaweeds (*Laminaria digitata*, *Ascophyllum nodosum*, *Pelvetia canaliculata*, *Fucus vesiculosus* and *Fucus spiralis*). A 'seaweed-like' aroma was, in general, the attribute with the highest score – which could be expected as it is the attribute most related to 'seafood-like'. Only Laminaria species extract was significantly different from all the others in terms of aroma, being the one with the strongest 'seaweed-like' aroma, and the mildest 'honey-like' aroma. Yamamoto et al. (2014) asked panellists to evaluate aroma attributes like animalic, floral, spicy, fatty, green note, marine-like, fresh (watery), powdery and leather-like, in *Ulva prolifera, Ulva linza* and *Monostroma nitidum*. They reported that, depending on the Japanese prefecture where the seaweed originated, seaweeds were evaluated differently in respect of its green-note, marine-like, fresh and powdery aroma. Some further studies analysed the aroma compounds produced by marine microalgae species (Isleten Hosoglu, 2018; Van Durme et al., 2013b) and flagellates (Guo et al., 2019), and concluded that they could be described by different aroma characteristics, but microalgae findings are not relevant to this research.

The volatile profiles of *H.elongata*, *U.pinnatifida*, *A.esculenta*, *L. japonica* fucoxanthin extract, *P.umbilicalis* and *P.palmata* were described, some for the first time in this study. This research, based on SPME GC-MS and TD GC-MS, has revealed that volatile compounds differ intensely in the five varieties of dried seaweed and the powder extract. We also reported key odorants of the different commercial brown and red seaweed samples. This work shows the potential of GC-MS and GC-O coupled with multivariate analysis (PCA) to discriminate between samples of different origins, based on their volatile profiles, and form the basis for future development of authentication methods and characterization of seaweed-containing products.

Little information is available on species such as *H. elongata*, U. pinnatifida, *P. umbilicalis*, and *P. palmata*, which are common brown and red species in abundance around the coastlines of Western Europe. For some species such as *A. esculenta* and *L. japonica* no details on their volatile profiles or odour characteristics have been published before. This study therefore addresses significant recent developments in the current understanding of the volatile profile of these seaweeds and the key aroma compounds that contribute to their unique odours. This characterization of volatiles in seaweeds may help to develop new products that are more acceptable to consumers who are less familiar with seaweed, especially as seaweeds are of growing importance in Western diets, and most recently as components of functional foods. The work has contributed to an understanding of volatile profiles in seaweeds and in particular our chemosensory knowledge of seaweed odour. Further work is required in relation to VOC changes that occur in the processing and storage of seaweed species as extracts and in their application in food in relation to sensory perception.

3.5. Limitations

Gas chromatography-Olfactometry alone does not allow a conclusion on the contribution of a single compound to the overall aroma. A reason for this is that the entire amount of a compound present in a sample is volatilized during GC-O, whereas, from a food, only the amount of an odorant present in the headspace above

the food is available for the nose receptors and thus is odour-active. Odour activity values (OAV) are helpful to understand the contribution of aroma compounds because they correlate quantitative data with odour thresholds in a matrix and thus address the influence of the matrix on the volatility of a given odorant (Dong et al., 2015). Different industries using different post-harvesting strategies can influence the volatile profile of the seaweeds as well as the extracts. A better understanding of how different food processes influence the volatiles profile of seaweed is essential, as it would be a powerful tool to tailor sensory properties and improve the final consumer acceptance of algae in food products (Stévant et al., 2018).

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Chapter 4

Chapter 4. Effect of salt reduction and inclusion of 1 % edible seaweed on the chemical, sensory and volatile component profile of reformulated frankfurters

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4.1. Abstract

The optimization of processed meats through salt replacement using edible seaweeds may reduce the risk of chronic disease through reduction in dietary sodium. We investigated the impact of the inclusion of four selected seaweeds (1 % w/w) in reformulated frankfurters in which salt addition and pork fat content was reduced by 50 % and 21 %, respectively, and where pork loin (*longissimus dorsi* muscle) was increased by 6 %, compared to a Control. Two different types of red (*Porphyra umbilicalis* and *Palmaria palmata*) and brown (*Himanthalia elongata* and *Undaria pinnatifida*) edible seaweeds were evaluated. The reformulated frankfurters containing added seaweed were lower in ash, higher in moisture, protein and darker in colour and had altered textural properties in comparison to the Control; mainly less hard and less chewy. The volatile and sensory profiles of the reformulated frankfurters differed from the Control. However, the reformulated frankfurters with the inclusion of *H. elongata* were the most promising, although further work is required to optimise the formulation.

4.2. Introduction

Irish consumers are concerned about dietary intake of fat, salt and cholesterol in pork products and are demanding healthier alternatives (Donoghue et al., 2008). There is a particular interest in the consumption of breakfast and frankfurter sausages in Ireland, but these products typically contain high fat and sodium levels with lipid profiles that diverge from dietary objectives for optimal health (Roohinejad

et al., 2017a). However, these products are of considerable economic importance and are widely accepted in certain sectors of the population (López-López et al., 2010). According to the National Adult Nutrition Survey (NANS) (McCarthy et al., 2017), 13 % of the Irish population are considered as 'processed pork indulgers', consuming five times more sausages, bacon and pudding than other meat consumer segments. Pork eaters derive 28 % of their energy intake from meat in their diet and have fat and salt intakes above that recommended for a healthy diet. Thus, from a public health perspective, there is a need to decrease salt (Trieu et al., 2017) and fat (Briggs et al., 2017) intake but this is difficult without comprising sensory quality.

Processed meat refers to meat (mainly pork or beef, but may also contain other meats or meat by-products) that have been transformed through salting, curing, fermentation, smoking or other processes to enhance flavour or improve preservation, and often contain high quantities of minced fatty tissues (e.g. sausages) (Domingo and Nadal, 2017). Processed meat products with enhanced composition have been formulated both by reducing the concentrations of salt, fat, cholesterol and nitrite (unhealthy compounds) and by promoting the presence of healthy compounds (e.g. bioactive compounds) (Arihara, 2006; Fernández-Ginés et al., 2005). Meat products adapted by the food industry using novel processing methods based on reformulation, reducing salt and fat and adding healthier components, are key strategies (e.g. modification of the animal's diet to produce raw material with healthy ingredients) under investigation (Beriain et al., 2018). Changes in formulation may have an impact on quality and palatability and must be accepted from the consumer perspective in order to attain general acceptance and market share. Frankfurters, burgers and patties, represent processed meat products where compositional modification is more easily attained to improve their nutritional value and health properties (Jiménez-Colmenero et al., 2010).

Seaweeds are good sources of nutrients and bioactive phytochemicals and have gained importance in Western diets, and most recently as components of functional foods (Gupta and Abu-Ghannam, 2011; Qin, 2018). All edible seaweeds offer considerable potential as natural sources of important components with many different biological activities (e.g. antioxidant (Y Zhao et al., 2018) and antihypertensive activities (Cofrades et al., 2010)), making them suitable candidates

as food ingredients for promoting health. Although reducing salt and fat in processed meats by the addition of seaweed may provide additional functional properties, such as nutritional (less calories and saturated fatty acids), physicochemical and textural properties (Roohinejad et al., 2017a), seaweed addition may also result in decreased consumer acceptability, due to the unique flavour of seaweed. The chemical composition of seaweeds is species dependent (Cofrades et al., 2010). The two red edible seaweeds, Nori (*Porphyra umbilicalis*) and Dulse (*Palmaria palmata*) contain higher quality proteins and proportionally larger amounts of all amino acids than the edible brown species, Sea Spaghetti (*Himanthalia elongata*) and Wakame (*Undaria pinnatifida*) (Cofrades et al., 2010). The lipid content of *H. elongata* is higher than that of *P. umbilicalis*, but similar to *U. pinnatifida* (Cofrades et al., 2010). The two brown species exhibit higher saturated fatty acid contents, than red species, but the main difference as regards to fatty acid profiles is the makeup of monounsaturated and polyunsaturated fatty acids (Cofrades et al., 2010). *H. elongata* contains the highest proportion of soluble and total dietary fiber, whereas *U. pinnatifida* is richer in insoluble fiber (Cofrades et al., 2010). All seaweeds are rich in ash content compared to land plants. *H. elongata*, *U. pinnatifida* and *P. umbilicalis* are also abundant in Na and K, macrominerals which in balance with Ca and Mg, help reduce high blood pressure (Cofrades et al., 2010). *H. elongata* also has a long history of safe use and acceptability in cooking (GarciaVaquero et al., 2017).

The incorporation of edible seaweeds through blending or ingredient substitution has been explored in only a minority of processed meat products (Choi et al., 2016; Cofrades et al., 2008; Jeon and Choi, 2012; Jiménez-Colmenero et al., 2010; López-López et al., 2009a), despite the fact that it is a relatively simple process and provides an effective measure to enhance meat quality (nutritionally and technologically speaking). A study on beef patties formulated with *H. elongata* found that texture and mouth-feel were improved without losing sensory quality (Cox and Abu-Ghannam, 2013). However, overall very little attention has been paid to the use of edible seaweeds as ingredients in processed meat products, especially in relation to sensory implications.

Therefore, the purpose of this study was to investigate the addition of common edible seaweeds (*H. elongata*, *U. pinnatifida*, *P. umbilicalis* and *P. palmata*) as a salt

replacer on compositional, physicochemical, volatile and sensory characteristics of reformulated frankfurters throughout storage (shelf life).

4.3. Material and methods

4.3.1. Meat and seaweed material

Pork loin from the *longissimus dorsi* muscle (90-95 % visual lean) was purchased along with pork fat from a local supplier (Staunton Foods Ltd., Timoleague, Co. Cork, Ireland). Pork's loin composition was 2.6 % fat, 73.3 % moisture, 1.7 % ash, 0.03 % sodium, 0.082 % sodium chloride (NaCl), 0.2 % glucose, 3.71 % N, 23.2 % protein, 1.09 % monounsaturated fatty acids, 0.41 % polyunsaturated fatty acids, 0.93 % saturated fatty acids. The meat and fat were vacuum-packed and stored at -18 °C until required for frankfurter production. Starch, farina (milled wheat), paprika, tomato powder, NaCl, phosphate, sodium caseinate, sodium ascorbate, sodium nitrite and carmine were obtained from AllinAll Ingredients Ltd. (Dublin, Ireland). Frankfurter spice and artificial cellulose casings (26 mm) were purchased from Fispak Ltd. (Dublin, Ireland).

Edible seaweeds (*H. elongata*, *P. umbilicalis* and *P. palmata*) were supplied by Wild Irish Seaweed Ltd. (Co. Clare, Ireland) and *U. pinnatifida* was sourced from Algamar (Pontevedra, Spain). All seaweeds were harvested along the west coast of Ireland or the north coast of Spain (*U. pinnatifida*). Irish Seaweed seaweeds were air-dried then dehumidified at Wild Irish Seaweed Ltd. facilities (Caherush Point, Spanish Point, Co. Clare, Ireland). Algamar seaweeds were dried at low temperature (<42 °C) at Polígono de Amoedo (Pazos de Borbén, Pontevedra, Spain). These seaweeds were chosen, because of their composition, potential health implications, abundance on Irish and Spanish coasts, relatively widespread consumption/acceptability, and suitability for use as ingredients in these processed meat products.

4.3.2. Sample preparation

Air-dried seaweeds were milled to a maximum size of 1 mm by means of a mechanical grinder. Minced samples were stored in a -20 °C freezer, for no longer than 15 days until their incorporation in the food product.

The formulation of control frankfurters were as follows: pork loin (65 %), pork fat (19 %), ice/water (10.15 %), starch (0.92 %), farina (0.92 %), frankfurter spice (0.5 %), paprika (0.5 %), tomato powder (0.25 %), NaCl (2 %), phosphate (0.25 %), sodium ascorbate (0.05 %), sodium caseinate (0.35 %), sodium nitrite (0.0075 %) and carmine (0.005 %). For the formulation of seaweed frankfurters, 1 % of salt was replaced with 1 % edible seaweed, and 4 % fat was replaced by more pork loin. Two independent batches of frankfurters with edible seaweeds were produced, following the same procedure.

The frozen meat and fat were cut into pieces and allowed to defrost at room temperature until they reached 4 °C. The meat and fat were then minced to a particle size of 3 mm through a mincer (Talsabell S.A., Valencia, Spain). The meat was weighed according to the formulations shown in Table 4.1 and placed in a bowl chopper (Seydelmann KG, Aalen, Germany). The required salt, preservatives (starch, farina, phosphate, sodium caseinate) and 3/4 of the water needed were added and mixed at low speed for 1 min; followed by adding and mixing the fat, seasoning (frankfurter spice, paprika and tomato powder) and the seaweed (only in the reformulated frankfurters) for further 2 min at high speed (3000 rpm). The remaining water and ice were finally added and mixed at high speed for ~45 sec, until a final temperature of 15 °C was reached. The frankfurter mix was then placed in a casing filler (Mainca, Barcelona, Spain) and stuffed into cellulose casings of 26 mm diameter, hand-linked (12 cm in length). The frankfurters were cooked at 100 °C in an electric steam-convection oven (Zanussi Professional, Italy) until the internal product temperature reached 74 °C, tested by a hand-held temperature probe (Testo 110, Lenzkirch, Germany). The temperature of the frankfurters was then held at 74 °C for 10 min. All samples were cooked at the same time, in duplicate, and segregated. Lastly, the frankfurters were allowed to cool down for 20 min, then vacuum sealed into

polyamide/polyethylene (PA/PE) laminate plastic bags (water vapour transmission rate of 2.8 g/m^2.day at 23 °C and 85 % relative humidity (RH), an O_2 and N transmission rate of 10 cm^3/m^2.day at 23 °C and 0 % RH and a CO_2 transmission rate of 150 cm^3/m^2.day at 23 °C and 0 % RH) and stored at 4 °C in a chill room. Frankfurters used for compositional, colour, texture and water holding capacity analysis, volatile analysis and sensory evaluation were stored for no longer than 10 days. Frankfurters used in the microbiological analysis and the lipid oxidation analysis were stored for 55 and 63 days, respectively.

Table 4.1. Frankfurter formulation.

	Control	***Himanthalia elongata***	***Undaria pinnatifida***	***Porphyra umbilicalis***	***Palmaria palmata***
Ingredients (g/100g)					
Pork loin	65.0	69.0	69.0	69.0	69.0
Pork fat	19.0	15.0	15.0	15.0	15.0
Seaweed	-	1.0	1.0	1.0	1.0
Water/Ice 50:50	10.25	10.25	10.25	10.25	10.25
Starch	0.92	0.92	0.92	0.92	0.92
Farina	0.92	0.92	0.92	0.92	0.92
Frankfurter spice	0.5	0.5	0.5	0.5	0.5
Paprika	0.5	0.5	0.5	0.5	0.5
Tomato powder	0.25	0.25	0.25	0.25	0.25
NaCl	2.0	1.0	1.0	1.0	1.0
Phosphate	0.25	0.25	0.25	0.25	0.25
Sodium caseinate	0.35	0.35	0.35	0.35	0.35
Sodium ascorbate	0.05	0.05	0.05	0.05	0.05
Sodium nitrite	0.0075	0.0075	0.0075	0.0075	0.0075
Carmine	0.005	0.005	0.005	0.005	0.005
Salt and fat levels (%)					
Salt	2.0	1.0	1.0	1.0	1.0
Fat	19.0	15.0	15.0	15.0	15.0

4.3.3. Compositional analysis

A reference meat sample with known moisture and fat content was analysed before measuring samples, to verify all analysis were correct. Moisture and fat content of 4 g of frankfurter were determined using the CEMSMART (moisture) and SMART Trac (fat) systems (CEM GmbH, Kamp-Lintfort, Germany) (Bostian et al., 1985), after having been homogenised using a Büchi Mixer B-400 (BÜCHI Labortechnik AG,Meierseggstrasse 40, Postfach, CH-9230 Flawil 1, Switzerland) and quickly transferred into a moisture proof bag to avoid evaporative loss. Both analyses were undertaken in duplicate. Moisture of 2 g of seaweed was determined by drying the sample in a preheated to 135 °C oven for 2 h (Association of Official Analysis Chemists International (AOAC), 1996). Fat content of 5 g of seaweed was determined using Foss Soxtec Avanti 2055 Manual system (Foss, Hillerød, Denmark). Protein content of frankfurters and seaweed was measured using the Kjeldahl method (Suhre et al., 1982). The homogenised sample was weighed accurately (0.5 g) into digestion tubes. Kjeldahl tablets (Thompson & Capper Ltd., Runcorn, Cheshire, UK) were added and the tubes placed into a heated digestion block (FODD, Tewwtor™ digestor, Hillerød, Denmark). The Kjeldahl distillation unit was a FOSS, Kjeltec™ 2100 (Hillerød, Denmark). The protein was calculated using a nitrogen factor of 6.25. All analysis were carried out in duplicate (Table 4.2).

The ash content of frankfurters and seaweed was determined in duplicate by a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) (Association of Official Analytical Chemists, 1923), which was pre-heated to 600 °C. Approximately 5 g of well blended sample was used for the ash analysis. The salt content of frankfurters and seaweed was carried out, in duplicate, by titration using silver nitrate. Silver nitrate solution was standardised against 0.100 % sodium chloride solution (0.1 N). Approximately 2 g of blended samples were put into muffle furnace to create ash. Ash was washed into conical flask with 20 ml distilled water and 2 ml of the chromate indicator was added. The flasks were potentiometrically titrated with 0.1 N silver nitrate from a clear yellow to an opaque light orange (+255 mV), after cooling to room temperature. Blank titration was performed using 20 ml distilled water. Sodium

content was estimated by a conversion factor (2.5 g of salt per 100 g is 1 g of sodium per 100 g) (Table 4.2).

4.3.4. Colour analysis

The frankfurter samples were allowed to stay at room temperature for 1 h before measurement (Table 4.2). Six readings were taken per sample. The cooked frankfurters were cut in half and the colour was measured according to the CIE L* a* b* colour system using a Minolta CR400 colorimeter (Minolta Camera Co. Ltd., Osaka, Japan) with an 11 mm-diameter aperture and D_{65} illuminant. Previous calibration was done by the CIE Lab colour space system using a white tile (C: Y=93.6, x=0.3130, y=0.3193) (Minolta calibration plate). The three coordinates of CIE L* a* b* represent the lightness of the colour (L^* = 0 yields black and L^* = 100 indicates diffuse white), its position between red/magenta and green (a^*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b^*, negative values indicate blue and positive values indicate yellow). Delta E 2000 formula was used to measure the visible difference between two colours, in this case between Control and reformulated frankfurters' colour. ColorTools Excel add-in was used to apply the formula. A smaller Delta E value (ΔE*) represents a closer colour match.

Table 4.2. Composition of frankfurters and seaweeds used in the formulation (per 100 g) and colour, texture analysis, water holding capacity and lipid oxidation values (TBARS) of frankfurters.

	Control	***Himanthalia elongata***	***Undaria pinnatifida***	***Porphyra umbilicalis***	***Palmaria palmata***	
	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	*P*-value
Composition						
Seaweeds						
% Moisture	N/A	16.31 ± 0.34^{a}	10.77 ± 0.03^{b}	8.99 ± 0.16^{c}	10.04 ± 0.41^{d}	<0.001
% Fat	N/A	1.42 ± 0.14^{ab}	2.30 ± 0.11^{c}	1.13 ± 0.03^{a}	1.69 ± 0.14^{b}	<0.001
% Protein	N/A	5.84 ± 0.23^{a}	9.02 ± 0.59^{b}	33.57 ± 1.14^{c}	19.96 ± 0.20^{d}	<0.001
% Ash	N/A	30.06 ± 0.23^{a}	51.18 ± 0.08^{b}	17.24 ± 0.25^{c}	25.64 ± 0.79^{d}	<0.001
% Salt	N/A	15.16 ± 0.25^{a}	36.77 ± 1.94^{b}	5.88 ± 0.13^{c}	16.34 ± 1.06^{a}	<0.001
Frankfurters						
% Moisture	59.57 ± 0.41^{a}	61.41 ± 0.19^{a}	61.31 ± 0.47^{a}	61.11 ± 0.72^{a}	61.90 ± 0.35^{a}	0.036
% Fat	11.92 ± 0.31^{a}	9.79 ± 0.36^{b}	9.70 ± 0.25^{b}	9.82 ± 0.18^{b}	9.33 ± 0.24^{b}	0.003
% Protein	18.61 ± 1.05^{a}	18.86 ± 0.89^{a}	18.99 ± 0.21^{a}	19.95 ± 0.53^{a}	19.62 ± 0.62^{a}	0.119
% Ash	3.12 ± 0.17^{a}	2.50 ± 0.07^{b}	2.77 ± 0.15^{ab}	2.41 ± 0.06^{b}	2.44 ± 0.07^{b}	0.005
% Salt	2.13 ± 0.05^{a}	1.37 ± 0.07^{b}	1.49 ± 0.08^{b}	1.19 ± 0.10^{b}	1.31 ± 0.10^{b}	0.001
Sodium (g)	0.853	0.548	0.595	0.476	0.522	-
Colour						

L*	70.93 ± 0.84^{a}	69.13 ± 0.62^{ab}	67.54 ± 5.33^{b}	64.79 ± 1.88^{c}	67.52 ± 2.30^{b}	<0.001
a*	10.16 ± 0.61^{a}	8.72 ± 0.54^{b}	5.33 ± 0.18^{c}	6.91 ± 0.48^{d}	8.01 ± 0.51^{e}	<0.001
b*	19.15 ± 0.42^{ab}	19.52 ± 0.54^{a}	18.41 ± 0.21^{b}	17.02 ± 0.70^{c}	18.31 ± 059^{b}	<0.001
ΔE*	N/A	2.014	5.355	5.677	3.265	-
Texture						
Hardness (N)	55.67 ± 3.98^{a}	44.29 ± 4.96^{b}	39.33 ± 2.26^{b}	41.85 ± 2.91^{b}	44.39 ± 5.51^{b}	0.003
Adhesiveness (N*mm)	-0.26 ± 0.10^{ab}	-0.23 ± 0.08^{ac}	-0.16 ± 0.08^{ad}	-0.10 ± 0.09^{cd}	-0.07 ± 0.04^{d}	0.005
Springiness (mm)	0.89 ± 0.02^{ab}	0.87 ± 0.02^{a}	0.90 ± 0.06^{ac}	0.92 ± 0.03^{bc}	0.92 ± 0.03^{bc}	0.009
Cohesiveness	0.77 ± 0.01^{a}	0.75 ± 0.01^{b}	0.77 ± 0.01^{ab}	0.77 ± 0.01^{a}	0.77 ± 0.01^{a}	0.005
Chewiness	38.53 ± 2.91^{a}	28.84 ± 3.23^{b}	26.85 ± 2.30^{b}	29.65 ± 1.83^{b}	31.44 ± 4.61^{ab}	0.006
Water holding capacity						
	88.03 ± 2.38^{a}	88.21 ± 2.25^{a}	86.73 ± 3.83^{a}	87.90 ± 2.87^{a}	87.62 ± 3.42^{a}	0.960
TBARS (mg MDA/kg meat) (day 63 storage)						
	0.459 ± 0.04^{a}	0.836 ± 0.07^{b}	0.515 ± 0.06^{a}	0.564 ± 0.08^{a}	0.531 ± 0.09^{a}	<0.001

M, mean; SE, standard error; N/A, not applicable; *P* <0.05 considered statistically significant; $^{a-e}$, means in the same row bearing different superscript letter denote statistical significant differences (*P* <0.05).

4.3.5. Texture analysis

Texture profile analysis (TPA) (Bourne, 1978) was used to characterize the texture of the product and was performed with a texture analyser XT3i (Stable Micro Systems, Surrey, UK). Three (10 mm × 10 mm) cylindrical slices of frankfurter were compressed to 40 % of their original height with a 35 mm diameter cylindrical probe (SMSP/35 Compression plate) in two cycles. Force–time deformation curves were derived with a 25 kg load cell applied at a constant crosshead speed of 1.5 mm/s. Texture profile parameters included: hardness (N), maximum force required to compress the sample; adhesiveness (N×mm), area under the abscissa after the first compression; springiness (mm), ability of the sample to recover its original form after deforming force was removed; cohesiveness (dimensionless), extent to which the sample could be deformed prior to rupture; and chewiness (N×mm), work required to masticate the sample before swallowing. All analyses were performed in triplicate and at room temperature.

4.3.6. Water holding capacity analysis

The water holding capacity was measured using the method described by Lianji and Chen (Lianji and Chen, 1989). Ten grams of frankfurter were wrapped in a cheese-cloth and placed in 30 ml centrifuge tubes lined with cotton wool at the bottom of each tube. The samples were centrifuged at 15,000 g for 10 min at 4 °C. After centrifugation, the cheese-cloth was removed and samples were reweighed. Water holding capacity was calculated using the following formula: % Water holding capacity = ((1-(B-A)/(B*M))*100, where B is the weight of sample before centrifugation, A is the weight of the sample after centrifuging, and M is the moisture content of sample determined using CEM SMART Trac system (CEM GmbH, Kamp-Lintfort, Germany).

4.3.7. Microbiological analysis

To ensure that frankfurter samples were safe for human consumption prior to sensory analysis, their microbiological quality was assessed by total viable count (TVC), after 1, 15 and 55 days of storage. Frankfurters were weighed (10 g) and placed into a stomacher bag. An initial 10-fold dilution was performed by adding 90 ml of sterilised maximum recovery diluent (MRD) (Oxoid, Baingstoke, UK). Then, the sample was stomached (Steward Stomacher 400 Lab Blender, London, UK) for 3 min, and the homogenate was 10-fold serially diluted using MRD solution. For the enumeration of TVC, 1 ml of appropriately diluted sample was inoculated on duplicated dry-total count plates (20 cm^2) (Nissui Pharmaceutical, Co. Ltd., Japan), followed by incubation at 30 °C for 48 h. All analysis was undertaken in duplicate. The limit of microbiological acceptability was set at 5 log colony-forming units (CFU)/g (Food Safety Authority of Ireland, 2019).

4.3.8. Lipid oxidation analysis

The lipid oxidation of frankfurters was assessed after 63 days of storage by the thiobarbituric acid (TBA) assay (G Siu and Draper, 1978). Five grams frankfurter samples were weighed into a container together with 25 ml distilled water and homogenised for 2 min using an Ultra Turrax T25 homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen, Germany). After adding 25 ml of trichloroacetic acid (TCA) (10 %), the mixture was shaken vigorously and centrifuged at 8,000 g for 15 min at 4 °C, and was filtered through a Whatman No.1 filter paper (Sigma-Aldrich, Darmstadt, Germany). 4 ml of sample and 1 ml of 0.06 M 2-TBA were added in screw cap test tubes. Assay blanks contained 2 ml distilled water and 2 ml 10 % TCA and 1 ml of 0.06 M TBA reagent. Test tubes were then placed in an 80 °C water bath for 90 min. The tubes were cooled at room temperature and vortex mixed to de-gas the samples. The absorbance of the filtrate was measured using a spectrophotometer at 532 nm against assay blank. The malondialdehyde (MDA) content of the samples was calculated using an extinction coefficient of 1.56 x 10^5

cm^{-1}. Results were expressed as TBA reactive substances (TBARS) in mg MDA/kg of meat. All analyses were undertaken in duplicate. The maximum acceptable limit was established at 1 mg MDA/kg meat (Warriss, 2010).

4.3.9. Sensory evaluation

Frankfurters were assessed by a 25 member panel in one session. All subjects were selected on the basis that they consumed and purchased frankfurters regularly. Sensory analysis was conducted in panel booths, which conform to International Standards (ISO, 2007) (International Organization for Standardization, 2007a). Five samples, cut in 3 cm length cylindrical slices, were presented to the panellists. Panellists were required to rinse with water before tasting each sample. Sample presentation order was randomised to prevent any flavour carryover effects (MacFie et al., 1989) and coded with a 3-digits code.

In the sensory acceptance test, assessors were asked to indicate their score on a continuous 10 cm line scale, ranging from 0 at the left to 10 at the right, for each frankfurter in duplicate. The scores were expressed in cm from the left baseline. The following attributes were evaluated: colour, liking of appearance, liking of aroma, liking of flavour, liking of texture and overall acceptability (hedonic attributes). After training, the assessors then participated in a Ranking descriptive analysis (RDA) (Richter et al., 2010). They were asked to assess the intensity of sensory attributes, such as tenderness, juiciness, salt taste, meat flavour, off flavour and seaweed flavour, which were also measured on a continuous 10 cm line scale. All samples were again presented in duplicate at ambient temperature.

4.3.10. Extraction and volatile compounds analysis

4.3.10.1. Standard solutions

The internal standard (2-phenyl-D5-ethanol), the check standards (1-butanol, dimethyl disulphide, butyl acetate, cyclohexanone and benzaldehyde) and external

standards (where available) were supplied by Sigma-Aldrich Ireland Ltd. (Arklow, Co. Wicklow, Ireland). Internal and External standard solutions were prepared at 0.1 % (v/v) in methanol and stored at -18 °C until required, but never longer than 3 months. The check standards solution was prepared with 124 µl 1-butanol, 96 µl dimethyl disulfide, 114 µl butyl acetate, 106 µl cyclohexanone and 96 µl benzaldehyde in 100 ml methanol. The standard solution mix (internal + check standards) was prepared as 100 µl of each solution in 10 ml distilled water daily as required. 10 µl of the standard solution mix were injected in the thermal desorption (TD) tubes with a syringe.

4.3.10.2. Thermal desorption

Ten grams of finely diced frankfurter (100 µl of internal standard was added at 50 ppm) were placed in stainless steel chamber inert coated pot and cooked in a Markes International Ltd. Micro-Chamber/Thermal Extractor (Llantrisant, UK) for 40 min at 72 °C with a nitrogen flow of 50 ml/min. Samples were analysed in triplicate. Extraction of volatile compounds was carried out while the frankfurters were cooking. Volatiles were trapped in a TD Tenax Carbograph tube (Markes International Ltd.) in series after a prepared sodium sulphate TD tube (Markes International Ltd.) dry packed with 1.5 g of sodium sulphate granules which is used as a moisture trap. The flow rate through the tubes was monitored to ensure that flow was not interrupted during the cooking process due to condensation of moisture in the extraction tubes. Control and reformulated samples were treated in an identical manner.

4.3.10.3. Gas chromatography-mass spectrometry

A Unity 2 Thermal Desorption Unit (Markes International Ltd.) was used to concentrate the volatiles and remove any excess moisture prior to direct transfer to the gas chromatography-mass spectrometry (GC-MS). The Unity 2 was operated with purge gas of nitrogen at 50 psi at a flow rate of 1.2 ml/min. Tubes were dry purged

for 2 min using a 1:20 split. A two-stage desorption was employed: the first stage desorption was performed at 150 °C and held for 5 min, the second stage desorption was performed at 300 °C and held for 5 min. A 1:10 split was used for final tube desorption. The trap used was a materials emissions trap and this was held at 30 °C during tube desorption with a gas flow of 50 ml/min. Prior to trap desorption, a 2 min pre-trap fire purge was performed with a 1:50 split. Trap desorption was performed at 30-300 °C at a rate of 24 °C/min and held for 5 min with 1:10 split.

The GC-MS system was an Agilent 7890A GC with an Agilent 5977B mass selective detector (MSD) (Agilent Technologies Ltd., Cork, Ireland). The column used was a capillary DB-624 UI (60m x 0.3mm x 1.8µm) (Agilent Technologies Ltd., Ireland) with helium as the carrier gas. The column temperature started at 40 °C held for 5 min, increased to 230 °C at 5 °C/min and held at 230 °C for 35 min. The total run time was 78 min. Injector temperature was set at 250 °C. Desorbed volatile compounds were injected in splitless mode with a consistent pressure of 23 psi. The mass spectra of volatile compounds were generated by a MS quadrupole detector with ionisation voltage of 70 eV, 3.32 scans/s and a scanning mass range of 35-350 amu. The ion source temperature was 220 °C and the interface temperature was set at 280 °C.

A set of check standards were run as part of each sample set and abundances were compared to known amounts to ensure that both the TD extraction and MS detection were within specification. Volatile compounds were identified by comparing their mass spectra to the NIST 2014 mass spectral library and an in-house library created using authentic external compounds with target and qualifier ions for each compound, using MassHunter Qualitative Analysis Software (Agilent Technologies, Palo Alto, CA, USA). Spectral deconvolution was also performed to allow the identification of untargeted and co-eluting compounds using Automatic Mass Spectral Deconvolution and Identification System (AMDIS) (http://chemdata.nist.gov/mass-spc/amdis/). Linear retention indices (LRI) were determined using the method by Van Den Dool and Kratz (van Den Dool and Dec. Kratz, 1963) and matched against peer reviewed publications (Corral, Salvador, Belloch, et al., 2015; Gallego et al., 2012; Gianelli et al., 2011). Positive identification was only attained once the mass spectral match reached >80 %, using external

standards where possible and when LRI were within 10 % of referenced values. The results were expressed in abundance values.

4.3.11. Data analysis

The compositional data, sensory evaluation data (sensory acceptance test and RDA), the data acquired by instrumental methods and TBARS values were evaluated by Bonferroni multiple comparison analysis (one-way ANOVA), using Statistical package for the social sciences (SPSS) software version 24 (IBM Corp., Armonk, NY), to determine if there were statistically significant differences (P <0.05) between different formulations. In addition, sensory evaluation data and instrumental measurements were further analysed using ANOVA partial least squares regression (APLSR). Regression coefficients were analysed by Jack-knifing to derive significance indicators for the relationships determined in the quantitative APLSR (data not shown). This further analysis was performed using the Unscrambler X Software, version 10.3 (CAMO ASA, Trondheim, Norway).

R software (RStudio Inc., Boston, MA) for statistical computing and graphics was used to analyse the volatile component profile and obtain a hierarchical clustering heatmap with the abundance values of volatile compounds derived from each frankfurter formulation. Principal component analysis was also carried out to aid the association of volatile compounds within the frankfurter samples.

4.4. Results and discussion

4.4.1. Physiochemical analysis

The moisture content of the reformulated samples was not statistically different from the Control (Table 4.2). The moisture content of frankfurters was inversely associated with fat and salt content, in that the lower fat samples correlated to higher moisture, and higher salt samples correlated to lower moisture content. Similar findings were obtained by Tobin et al. (Tobin et al., 2013) on pork

breakfast sausages. Lower moisture contents also correlated with lighter colour of frankfurters, and *vice versa*, matching results by Ahmed et al. (Ahmed et al., 1990), where the colour in sausages was generally influenced by fat content and moisture. The water holding capacity of all frankfurter samples was at a relatively high level (>86.0 %), although no significant differences were recorded (Table 4.2). Water holding capacity is the ability of food to hold its own or added water during the application of force, pressure, centrifugation, or heating. It is also the ability of food to retain water against gravity and has shown to play a major role in the formation of food texture (Gyawali and Ibrahim, 2016). The water binding property of myoglobin is expected to decrease in meat products when salt content is reduced, because salt plays an important role in solubilising and improving functionality of myoglobin (Desmond, 2006). However, this was not the case in this study. The salt contents of the seaweed preparations are also provided in Table 4.2. It is evident that the salt contents are significantly different (P <0.001), with highest levels in the *U. pinnatifida* sample. These salts levels were quite different to those attained in a previous study (Pereira, 2011) and likely highlight differences in preparation. These seaweeds also naturally contain high levels of minerals, such as potassium, magnesium, zinc, iron and calcium that can all add to the "saltiness" perception and play a role in flavour development (Hotchkiss, 2010). The salt content of reformulated frankfurters was significantly reduced (P <0.001) (Table 4.2). Salt reduction exceeded 30 % for all products but was highest in the reformulated frankfurters containing red seaweeds (*P. umbilicalis* and *P. palmata*).

All the reformulated frankfurters have statistically significantly (P <0.05) lower fat contents than the Control (Table 4.2). Although the fat contents in the reformulated frankfurters were not reduced enough to be classified as a reduced fat product, fat content reduction ranged from 17.6-21.7 %. The fat content of each seaweed preparation is also provided in Table 4.2, and were similar to values obtained in a previous study (Pereira, 2011).

The two red seaweeds (*P. umbilicalis* and *P.palmata*) had a significantly higher levels (P <0.001) of protein than the two brown seaweeds (*H. elongata* and *U. pinnatifida*), but this was not evident in the reformulated frankfurters (Table 4.2). The protein content in the reformulated frankfurters was statistically similar to the

Control. Seaweed protein contents are highly variable with a previous study finding protein contents ranging from 5 to 39 % in *H.elongata*, *U. pinnatifida*, *P. umbilicalis* and *P. palmata* (Pereira, 2011). This same study also found variable ash contents in these same seaweeds from 12 to 40 % (Pereira, 2011). The ash contents of the seaweed samples in this study varied from 17.24 to 51.18 %, with highest levels in the *U*. *pinnatifida* preparation (Table 4.2). The ash content of the reformulated frankfurters was lower than that of the Control samples (Table 4.2), possibly due to the salt reduction. Similar findings were obtained by Fellendorf et al. (Fellendorf et al., 2017), who observed an increase in ash content with an increase of salt content in black pudding. Frankfurters reformulated with *U. pinnatifida* accounted a higher percentage of ash, while frankfurters reformulated with *P. umbilicalis* accounted the lowest percentage.

Significant colour (P <0.001) differences were evident between Control and reformulated frankfurters (Table 4.2). L* values were found to be significantly (P <0.001) lower (darker) in all the reformulated frankfurter samples except those containing *H. elongata*. The reformulated frankfurters also had significantly lower (P <0.001) a* values (less magenta or more green) than the Control, and this was especially evident in the reformulated frankfurter with added *U. pinnatifida*. The b* values were also significantly lower (P <0.001) in the reformulated frankfurters (more blue, less yellow), except for those with added *H. elongata*. However, previous studies have reported that the addition of 3.3 % *H. elongata* reduced the L*, a* and b*, values in frankfurters and the incorporation of *H. elongata* (2.5 %) and *U. pinnatifida* (2.5 %) also reduced L*, a* and b* values in gel/emulsion systems (Cofrades et al., 2008; LópezLópez et al., 2009c). The addition of seaweeds to frankfurters, especially *P. umbilicalis* and *U. pinnatifida* impacted on the overall colour, as reflected by the ΔE* values (total colour difference) (Table 4.2). Changes in colour are likely directly due to the seaweed, but also possibly due to Maillard reactions, which are boosted in some pork products by the addition of seaweeds (Moroney et al., 2015).

The reformulated frankfurters had significantly lower hardness (P = 0.003) and chewiness (P = 0.006) values than the Control. López López et al. (López López et al., 2009b) showed that a 5 % addition of seaweed significantly increased the

chewiness and hardness in reformulated frankfurters. The contradiction between these results is likely to relate to differences in seaweed preparation and the amount added. Cohesiveness was reduced (P = 0.005) in reformulated frankfurters with the added *H. elongata*. Springiness increased (P = 0.009) in reformulated frankfurters with added *P. umbilicalis* or *P. palmata* and adhesiveness statistically increased (P = 0.005) in the reformulated frankfurters with added *P.umbilicalis* or *P. palmata* (Table 4.2).

4.4.2. Microbiological analysis

The microbiological quality of all the frankfurters at day 1 of storage was below 10 CFU/g (Fig. 4.1) and remained below the limit of acceptability (Log 5 CFU/g) at 15 days of storage. However, the reformulated frankfurter samples containing *U. pinnatifida* had a higher TVC than all other samples (P = 0.027) at day 15. The limit of acceptability (>5 CFU/g) was exceeded in all frankfurter samples after 55 days of storage, and again the reformulated frankfurter samples with *U. pinnatifida* had significantly higher TVC than other samples (P <0.001). Therefore, the shelf life from in the reformulated frankfurter samples with added 1 % *U. pinnatifida* is less than all other samples, and these results are in agreement with the microbiological analysis carried out by López López et al. (López-López et al., 2010) in beef patties. The estimated shelf life of the control samples was 50 days, and it was similar to the shelf life of frankfurter samples in a study by O'Neill (O' Neill et al., 2018), in which the same control formulation was used. Salt has a preservative effect in processed meat by lowering water activity, which helps control microbial growth. The reduction thereof can then have a negative impact on the shelf life. However, the estimated shelf life of reformulated frankfurters with added *H. elongata*, *P. umbilicalis* or *P. palmata* was similar to the Control.

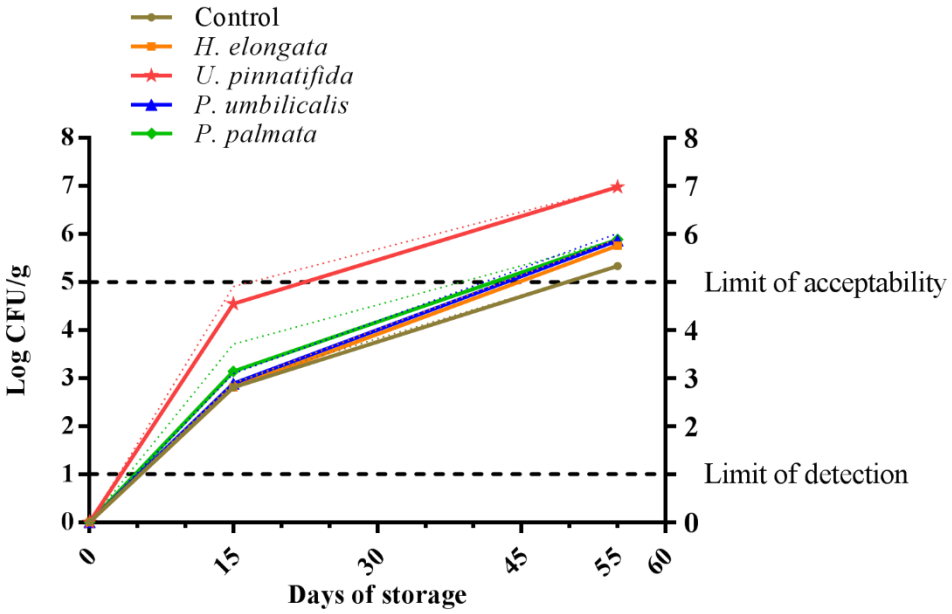


Figure 4.1. TVC changes across storage period of frankfurters. Dot lines represent standard deviations.

4.4.3. Lipid oxidation

The TBA assay for lipid oxidation generated TBARS values (Table 4.2) were below the maximum acceptable limit of 1 mg MDA/kg meat for all samples, at day 63 of storage. The frankfurter samples formulated with *H. elongata* had significantly (P <0.05) higher TBARS values which may indicate a greater susceptibility to lipid oxidation or reduced antioxidants in comparison to the other samples. Seaweeds can reduce lipid oxidation due to their antioxidant content but may also affect the susceptibility to lipid oxidation due to their polyunsaturated fatty acids content. The addition of brown seaweed polysaccharides was demonstrated to reduce lipid oxidation in pork patties during cooking, owing to the formation of potential antioxidant melanoidins (Maillard reaction products) (Moroney et al., 2015). The addition of 40 % *H. elongata* powder to beef patties also exhibited a high antioxidant capacity, and yet such high levels of addition were deemed acceptable in terms of sensory quality (Cox and Abu-Ghannam, 2013). We did not find a direct comparable study measuring lipid oxidation in frankfurters formulated with seaweed; however,

a study by Francisco Jiménez-Colmenero et al. (Jiménez-Colmenero, 2007) found that if olive oil was used to partially replaced pork back fat in fermented sausages and frankfurters, no oxidation issues were detected. We can conclude that the rate and extent of lipid oxidation in the reformulated frankfurters in this study is not limiting shelf life.

4.4.4. Sensory consumer evaluation

Sensory evaluation and instrumental data are presented in the PLSR plot in Fig. 4.2 with the corresponding scores and ANOVA *P*-values in Tables 4.2 and 4.3.

4.4.4.1. Sensory and hedonic attributes

P. umbilicalis and *P. palmata* frankfurters in particular are highly positively correlated with each other in terms of sensory properties, while *U. pinnatifida* frankfurters were somewhat also correlated. Both *P. umbilicalis* and *P. palmata* frankfurters are negatively correlated in comparison to the Control (Fig. 4.2).

In a study by Tobin et al. (Tobin et al., 2012a), frankfurters with salt contents lower than 1.5 % had a significant negative impact (P <0.05) on consumer acceptance. However, in a subsequent study by Fellendorf et al. (Fellendorf et al., 2015) white pudding products containing 0.6 % sodium were accepted (P <0.05) without using ingredient replacers. All of the seaweeds assessed in this study changed the sensory profile of frankfurters. Some of those changes were deemed positive and others mainly negative by sensory panellists. Frankfurters containing *H. elongata* were the most accepted and closest to the Control (Table 4.3). The overall acceptability was mainly influenced by the seaweed flavour rather than by a reduction of salt content, as also evident in the study by Fellendorf et al. (Fellendorf et al., 2016) where the impact of seaweed-based ingredients was perceived negatively (P <0.001) on white pudding flavour. Liking of appearance and liking of aroma of the Control samples were rated significantly higher than all other reformulated frankfurter samples (Table 4.3), highlighting the negative impact of seaweed addition on the appearance and

aroma perceptions. This negative impact was lowest in the *H. elongata* sample (Fig. 4.2). Both liking of flavour and liking of texture followed a similar trend to liking of appearance and liking of aroma, except that reformulated samples with *H. elongata* were not rated significantly lower than the Control. Sensory evaluation scores were lowest in reformulated frankfurters with added *P. palmata* (Table 4.3).

Consumers perceived that the reformulated frankfurters were darker in colour than the control in the order of *P. umbilicalis* > *U. pinnatifida* > *P. palmata* > *H.elongata* (Tables 4.2 and 4.3). Those consumer perceptions were confirmed by the ΔE* values (Table 4.2), which showed a bigger difference in colour between the Control and the frankfurters with added *U. pinnatifida*, *P. umbilicalis* and *P. palmata*, than with those containing *H. elongata*. Colour intensity was strongly related to liking of appearance, the samples with a lower rating of appearance had a higher colour intensity, which in turn adversely impacted on the overall acceptability.

Texture (fine to coarse), tenderness (tender to tough) and juiciness (dry to juicy) are very important texture attributes. A significantly negative (P <0.01) correlation between tenderness and *U. pinnatifida* and a significantly positive (P <0.01) correlation between tenderness and *P. palmata* was observed (Fig. 4.2). This was also reflected in sensory evaluation scores in Table 4.3. Overall seaweed addition generally increased the coarseness and slightly increased the tenderness of the reformulated frankfurters. Frankfurters containing *P. umbilicalis* and *P. palmata* had lower sensory scores (Table 4.3) and were significantly (P <0.01) negatively correlated to juiciness. Fat is considered essential in providing juiciness and hardness in meat products, as well as forming stable emulsions, reducing cooking loss, improving water holding capacity and binding properties (Choi et al., 2010). Sausages with a higher fat content are perceived juicier and easier to chew, but have less intense colour and are less shiny, compared to those with a lower fat content (Ventanas et al., 2010). Similar findings on the effect of fat levels on sensory scores were found for low fat frankfurters (Choi et al., 2010); and for patties (Mohammad Nisar et al., 2010; Serdaroglu, 2006; Tobin et al., 2012b). Ruusunen et al. (Ruusunen et al., 2003) also found that higher salt content frankfurters resulted in increased firmness and juiciness, similar to results of this study. The correlations between juiciness and higher salt levels are most likely due to the greater extraction of

myofibrillar proteins, thereby leading to enhanced moisture binding. On the contrary, Ventanas et al. (Ventanas et al., 2010) found sausages with a higher salt content were perceived as less juicy, harder to chew and having a more intense colour than those with a lower salt content.

Additionally, the regression coefficient *P*-values (not shown) demonstrated a negative correlation between all four reformulated frankfurters and salt taste, with the latter statistically significant for *P. umbilicalis* ($P < 0.01$) and *P. palmata* ($P < 0.001$). Frankfurters with added *H. elongata* scored lowest (Table 4.3). Salt taste is directly correlated to salt content; therefore, as expected, the salt taste of Control samples scored significantly higher than the reformulated samples. No significant difference in salt content was found between the reformulated frankfurters. The flavour profile of frankfurters is highly influenced by salt taste and meat flavour (Ruusunen et al., 2001). In this study, meat flavour was negatively correlated to frankfurters containing *P. umbilicalis* and *P. palmata*. However, both frankfurters containing *U. pinnatifida* or *H. elongata* had positive ($P < 0.01$) correlations to meat flavour perception (Fig. 4.2). On the other hand, the meat flavour of the Control samples scored significantly higher than the reformulated samples. This result is in agreement with Tobin et al. (Tobin et al., 2012a), who concluded that meat flavour in frankfurters is positively related to fat content. The positive correlation between the higher fat content and the perceived meat flavour by consumers may be explained by the fact that fat is a flavour enhancer and thus contributes to the overall flavour (Ruusunen et al., 2001). Fat also interacts with other ingredients and helps to develop texture, mouth feel and provides a lubricating effect in processed meats (O'Sullivan, 2016).

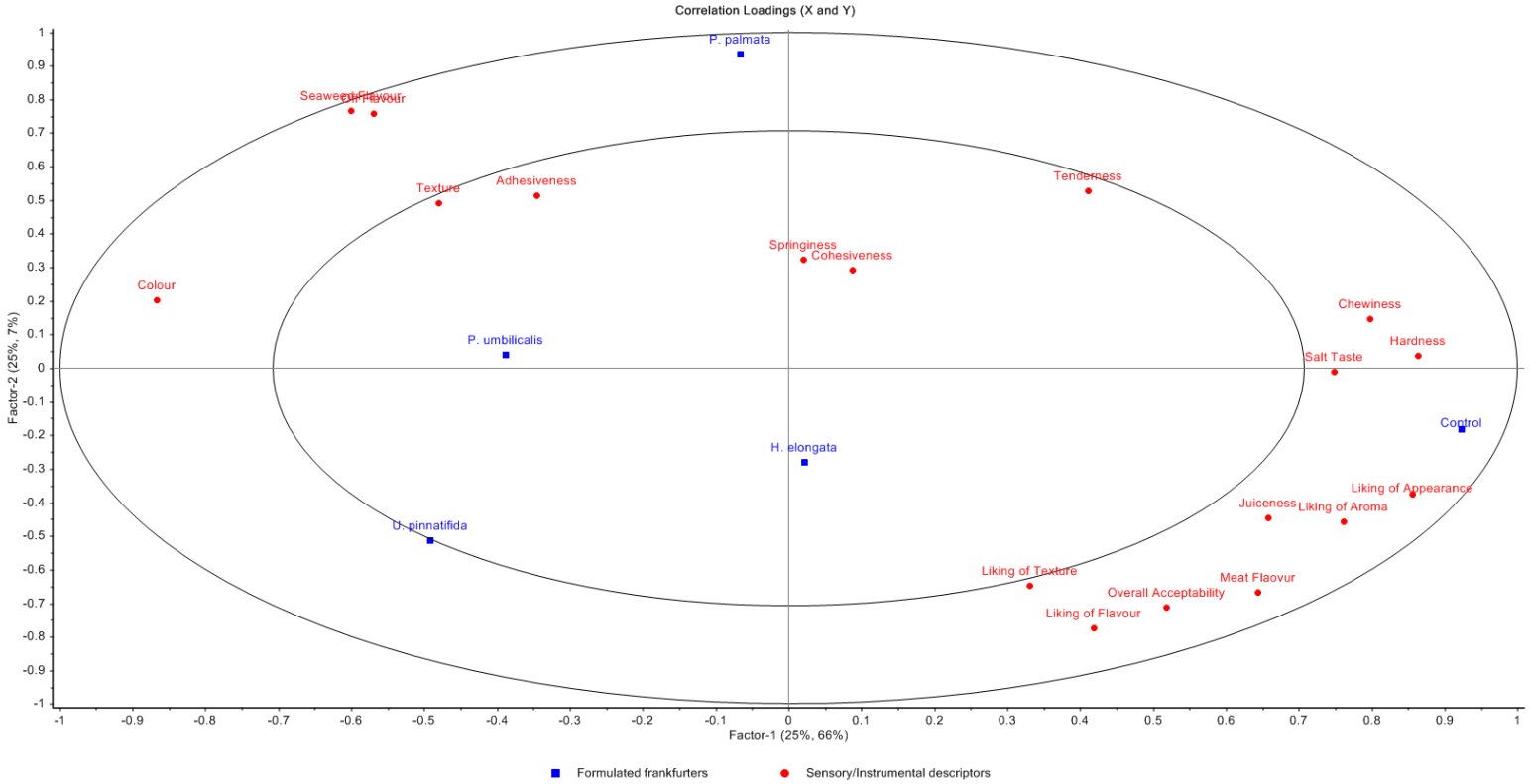


Figure 4.2. Partial least squares regression (PLSR) correlation loadings plot for frankfurter formulations with different salt replacers (Dulse = *Palmaria Palmata*; Nori = *Porphyra Umbilicalis*; Wakame = *Undaria pinnatifida*; Sea Spaghetti = *Himanthalia elongata*). Shown are the X- (sensory and instrumental data) and Y- (treatment groups) variables for the first two principal componentes (PC1 and PC2) for the • (red) = sensory descriptors and instrumental variables, and ▪ (blue) = control and reformulated frankfurters. The concentric circles represent 100 % (outer) and 50 % (inner) explained variance.

The diversity of volatiles compounds present in seaweeds produce different odours which contribute to the 'seaweed flavour' attribute in this study. Characteristic seaweed flavour was only detected in the reformulated frankfurters, which influenced overall acceptability of these products. The inclusion of *U. pinnatifida*, *P. umbilicalis* and *P. palmata* had a significant (P <0.001) impact on the seaweed flavour of the reformulated frankfurters (Fig. 4.2). Reformulated frankfurters containing *P. palmata* or *P. umbilicalis* were found to have the strongest seaweed flavour, followed those formulated with *H. elongata* and *U. pinnatifida* (Table 4.3). Seaweed flavour was also found to be a factor that reduced the acceptability of the reformulated frankfurters (Jiménez-Colmenero et al., 2010; López López et al., 2009b). An intense seaweed flavour is a rather unfamiliar flavour to Western consumers. In any event, the unfamiliar flavour intensity to western cultures can always be limited by selecting less strongly flavoured seaweed or reformulating via selected seasoning. Even so, for population sectors to whom seaweed flavour is familiar, the characteristic flavour of frankfurter formulated with seaweed would not be an obstacle. Seaweed flavour was also an important factor that contributed to the perception of off-flavour. Reformulated frankfurters scored significantly higher than Control the samples for off-flavour. Off-flavour was negatively correlated to frankfurters containing *H. elongata* and *U. pinnatifida* and positively and significantly correlated to frankfurters with *P. umbilicalis* (P <0.001) and *P. palmata* (P <0.001) (Fig. 4.2). *P. palmata* frankfurters were percieved to have the highest off-flavour (Table 4.3) and thus not accepted by consumers. Similarly, in a study by Jimenez Colmenero et al. (Jiménez-Colmenero et al., 2010) panellists were able to detect the addition of *H. elongata* seaweed as an off-flavour (seaweed-like).

Table 4.3. Sensory evaluation scores for the different frankfurter formulations with edible seaweeds.

Attribute	Control	*Himanthalia elongata*	*Undaria pinnatifida*	*Porphyra umbilicalis*	*Palmaria palmata*	
	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	*P*-value
Sensory acceptance test (hedonic attributes)						
Overall acceptability	6.27 ± 1.63^{a}	5.78 ± 1.33^{ab}	5.17 ± 1.69^{b}	5.11 ± 1.66^{b}	3.90 ± 2.03^{c}	<0.001
Liking of appearance	6.43 ± 1.94^{a}	5.37 ± 1.43^{b}	4.91 ± 2.13^{bc}	4.23 ± 2.31^{d}	4.57 ± 1.84^{cd}	<0.001
Liking of aroma	5.57 ± 1.77^{a}	4.63 ± 1.81^{b}	4.39 ± 1.89^{b}	4.31 ± 1.85^{b}	3.95 ± 1.84^{b}	<0.001
Liking of flavour	6.47 ± 1.55^{a}	5.99 ± 1.33^{a}	5.44 ± 1.88^{a}	5.40 ± 1.85^{a}	3.63 ± 2.46^{b}	<0.001
Liking of texture	5.74 ± 2.34^{a}	5.64 ± 1.54^{a}	5.45 ± 1.72^{ab}	5.03 ± 1.94^{ab}	4.52 ± 1.^{97b}	0.014
Colour	2.82 ± 1.52^{a}	4.24 ± 1.38^{b}	5.90 ± 2.11^{c}	7.54 ± 1.09^{d}	5.61 ± 1.63^{c}	<0.001
Ranking Descriptive Analysis (sensory attributes)						
Tenderness	4.86 ± 2.30^{a}	4.13 ± 1.84^{ab}	3.90 ± 1.80^{b}	4.69 ± 1.95^{ab}	4.84 ± 1.93^{a}	0.006
Juiciness	4.99 ± 2.00^{a}	4.46 ± 1.84^{ab}	4.12 ± 1.95^{ab}	3.97 ± 2.02^{b}	3.75 ± 1.98^{b}	0.003
Salt taste	5.76 ± 2.17^{a}	4.02 ± 2.03^{b}	4.43 ± 2.25^{b}	4.34 ± 2.02^{b}	4.55 ± 2.26^{b}	<0.001
Meat flavour	6.13 ± 1.95^{a}	4.90 ± 1.69^{b}	4.73 ± 1.77^{b}	4.62 ± 1.97^{b}	3.48 ± 2.03^{c}	<0.001
Seaweed flavour	0.68 ± 0.92^{a}	3.19 ± 2.26^{b}	3.01 ± 2.31^{b}	4.36 ± 2.65^{c}	6.52 ± 2.47^{d}	<0.001
Off flavour	1.11 ± 1.67^{a}	2.16 ± 1.76^{b}	2.56 ± 2.36^{b}	2.95 ± 2.51^{b}	4.46 ± 2.89^{c}	<0.001
Texture	3.85 ± 2.53^{a}	4.65 ± 1.80^{ab}	4.14 ± 2.00^{a}	5.75 ± 2.21^{c}	5.22 ± 2.19^{bc}	<0.001

M, mean; SE, standard error. *P* <0.05 considered statistically significant; $^{a-d}$, means in the same row bearing different superscript letter denote statistically significant differences (*P* <0.05). Hedonic attributes were scored on a 10 cm-line liking scale and sensory attributes were scored on a 10 cm-line intensity scale.

4.4.4.2. Sensory texture profile analysis

The Control frankfurters significantly differed (P <0.001) from reformulated frankfurters in terms of hardness and chewiness, except for those with added *P. palmata* (Table 4.3). Controls also significantly differed (P <0.001) from *H. elongata*-containing frankfurters for cohesiveness (Table 4.3). Adhesiveness was statistically significantly different (P <0.001) between the Control and those with added *P. umbilicalis* or *P. palmata* (Table 4.3). Springiness was statistically different (P <0.001) between the reformulated frankfurters with added *H. elongata*, *P.umbilicalis* or *P. palmata* (Table 4.3).

4.4.5. Volatile compounds

A total of 22 terpenes, 21 alcohols, 17 aldehydes, 8 esters, 11 ketones, 4 phenylpropenoids, 4 sulphur compounds, 3 phenols, 1 pyrazine, 1 furan, 1 benzene and 1 pyrrole were detected in the Control and reformulated frankfurters (Table S-4.1). Quite a lot of diversity existed between the volatile profiles, especially in relation to levels of abundance of individual volatiles. Terpenes and esters attained their highest levels in the reformulated frankfurters containing *H. elongata*; Ketones, phenylpropanes and phenols the most abundant volatile classes in reformulated frankfurters containing *P.* umbilicalis. Alcohols, aldehydes and sulphur compounds were at greatest abundance in reformulated frankfurters containing *P. palmata*; while the reformulated frankfurters containing *U. pinnatifida* did not any predominant class of volatile compounds. High amounts of o-cymene, ß-phellandrene, 4-methyl-2-pentanol, γ-terpinene, α-thujene, 3-carene, β-pinene and α-pinene were present across all samples, while low levels of 2-undecenal, pyrrole, methyl valerate and ethyl ether were also present in all samples. 4-thujanol; (Z)-p-mentha-2,8-dien-1-ol; alpha-terpinyl acetate; methional; pyrrole; octanal; propanal, 2-methyl and 2-butanone were at lower levels in all four reformulated frankfurters compared to Control. It is apparent from the heatmap (Fig. 4.3) that the reformulation of frankfurters influenced the abundance of volatile compounds.

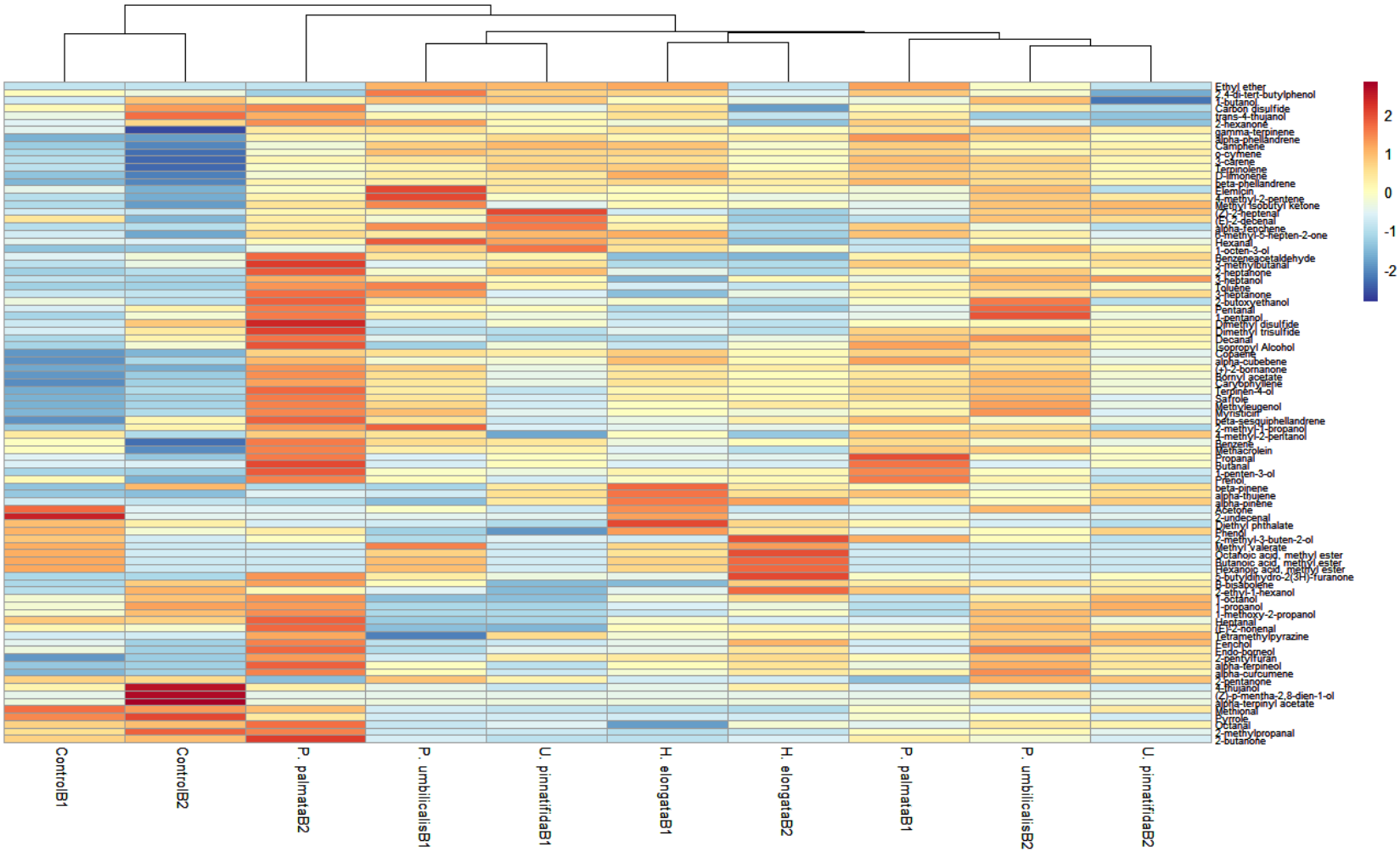


Figure 4.3. Hierarchical clustering heatmap of volatile compounds derived from frankfurters produced with different edible seaweeds in two batches. The abundance of the 50 most contributing volatile compounds is reflected in a degraded scale of colour: from red (higher abundance) to blue (lower abundance). B1, batch 1; B2, batch 2.

Alpha- and ß-pinene (terpene), prenol (phenol), 2-ethyl-1-hexanol (alcohol), butanal and propanal (aldehydes) and dimethyl trisulfide (sulphur compound) were strongly correlated to reformulated frankfurters with *H. elongata* (Fig. 4.2). Elemicin (phenylpropane), pentanal and hexanal (aldehydes), 3-heptanol and 1-butanol (alcohols), and methyl isobutyl ketone (ketone) were strongly correlated to reformulated frankfurters with *P. umbilicalis*. Isopropyl alcohol and 1-penten-3-ol (alcohols) and α-thujene and α-cubebene (ketones) were strongly correlated to reformulated frankfurters with *P. palmata*; while reformulated frankfurtes with *U. pinnatifida* strongly correlated to ß-bisabolene (terpene), benzene, 4-methyl-2-pentanol and 1-octen-3-ol (alcohol), (Z)-2-hepatanal and benzeneacetaldehyde (aldehydes) (Fig. 4.2).

The characteristic volatile compounds of some edible seaweeds have been analysed by GC-MS, which found that their volatile profile depend on the species, its geographical origin, processing method and environmental conditions (Watanabe et al., 2014). Some studies found that brown seaweeds produce sulphur compounds, C_{11}-hydrocarbons and diterpenes while red seaweeds are known to produce more halogenated compounds containing bromine and iodine, which contribute to a sweet note in the aroma of this specie of seaweeds (Leite Santos and Narain, 2018). Similar findings were obtained in this study, with terpenes having the highest abundance in the reformulated frankfurters containing the brown seaweeds (*H. elongata* and *U.pinnatifida*). On the contrary, no specific halogen compounds were found in the reformulated frankfurters containing red seaweeds.

In terms of volatile profiles, all reformulated frankfurters in batch 1 differed from the reformulated frankfurters in batch 2, highlighting that volatile profiles are more easily impacted by small changes in composition and processing in products such as frankfurters with multiple ingredients and extensive processing. The volatile profiles of *P. palmata* reformulated frankfurters differed most between batches (Fig. 4.3). None of the other analysis undertaken, showed evidence of batch variation.

The volatile compounds making the greatest contribution to differences between samples were o-cymene, camphene, 3-carene, β-phellandrene, terpinolene, 2-methyl-propanal, D-limonene, copanene, (+)-2-bornanone, bornyl

acetate, terpinen-4-ol, caryophyllene, safrole and alpha-curcumene (mostly terpenes) (Fig. 4.4). It is likely that many of these compounds are directly related to the seaweeds as the volatile profile of seaweeds can be very diverse (Maruti et al., 2018). Therefore, due to the strong marine flavour profile of seaweeds, it is difficult to determine whether changes in the volatile profile are due to a modification of the flavour profile through the addition of characters that are typical of seaweeds (e.g., marine, seaweed, salty, sulphur) or to a modification of the flavour profile through enhancement or potentiation of flavour characters typical of the frankfurters recipe to which the seaweed has been added.

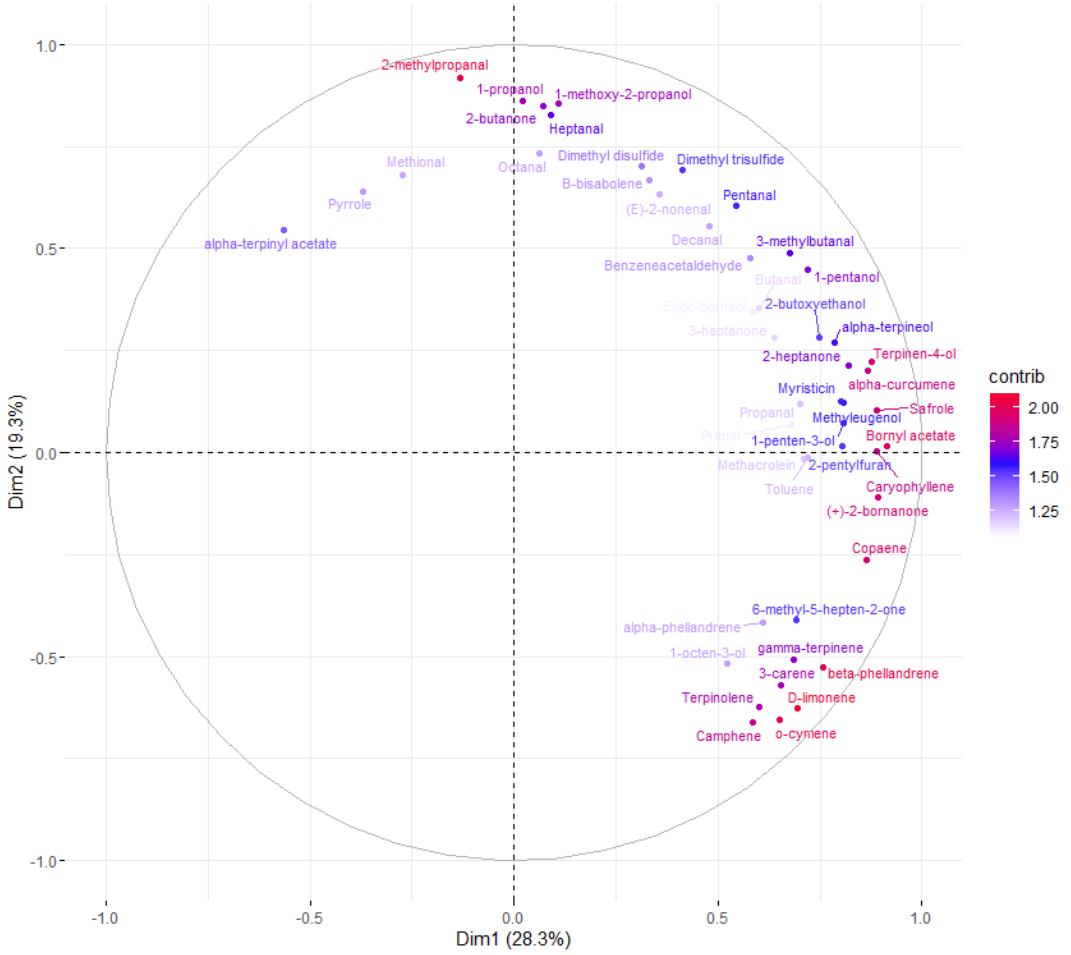


Figure 4.4. Principal component analysis (PCA) plot for the volatile compounds making the highest contribution to the differences between the frankfurters reformulated with different salt replacers (Dulse = *Palmaria palmata*; Nori =

Porphyra umbilicalis; Wakame = *Undaria pinnatifida*; Sea Spaghetti = *Himanthalia elongata*) and controls. The contribution of the 50 most determining volatile compounds is reflected in a degraded scale of colour: from red (higher contribution) to fade purple (lower contribution).

4.5. Conclusion

The inclusion of seaweed and reduction of salt and fat impacted on the sensory and physiochemical properties of frankfurters. Significant differences in colour, liking of appearance, aroma, flavour and texture attributes were evident between the samples. The overall acceptability of reformulated frankfurters containing seaweed was greatly influenced by the type of added seaweed, with reformulated frankfurters containing *H. elongata* being the most accepted. Inclusion of seaweed greatly influenced the abundance of volatiles compounds, but further research is required to determine the impact of individual volatile compounds on the sensory properties.

The addition of *U. pinnatifida*, *P. umbilicalis*, *P. palmata* and especially *H. elongata* have the potential to improve the nutritional quality through mainly salt reduction. It may require further processing of seaweed prior to addition, optimization of dose rates, combinations of seaweeds and/or highlighting potential nutritional benefits before such products are accepted by consumers unfamiliar with seaweeds in their diet.

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Chapter 5

Chapter 5. Optimization of a high-capacity sorptive extraction gas chromatrography-mass spectrometry method for untargeted volatile profiling and for the quantification of targeted lipid oxidation volatile compounds in raw beef patties

This chapter is under review in a peer reviewed journal.

5.1 Abstract

Lipids cause both desirable and undesirable aromas in meat. The aim of this study was to develop a gas chromatography-mass spectrometry (GC-MS) method using headspace high capacity sorptive (HiSorb) extraction for both untargeted volatile profiling and for quantification of target lipid oxidation compounds (LOC) in raw beef patties over refrigerated storage in modified atmosphere packaging (MAP) for 21 days. Optimal HiSorb extraction conditions (1g of sample extracted for 180 min at 40 °C) were achieved using 9 target LOC spiked into the beef patties using response surface methodology. In total 49 volatiles were identified. The volatile profile changed over storage, thiobarbituric acid reactive substances (TBARS) values increased (P <0.001), colour changed (P <0.002) and microbial counts were within acceptable guidelines. The HiSorb thermal desorption (TD) GC-MS method demonstrated good linearity, precision, and interday and intraday repeatability for all target LOC. However, 1-hexanol and 2-heptanone were identified as the best indicators of lipid oxidation for these samples based on lowest limits of detection and quantification, and increase over storage.

5.2 Introduction

Lipids are important components that impact the flavour and aroma of meats and meat products and are responsible for many desirable characteristics (Amaral et al., 2018a). However, lipid oxidation is one of the main factors associated with the

reduction in quality and shelf life of meat products, adversely impacting sensory (colour and flavour) perception (Lee et al., 2020).

Meat products are susceptible to lipid oxidation in the presence of oxygen, light, heat, and free radicals (Lee et al., 2020), resulting in the oxidation of unsaturated fatty acids and phospholipids (Cheng, 2016) to volatile organic compounds (VOC). Oxidation does not just impact lipids, but also proteins and vitamins, resulting in a decrease in nutritional quality, alteration of colour and the development of off-flavours, all of which adversely impact consumer acceptability (Domínguez et al., 2019; Kumar et al., 2015).

There is an increasing need to monitor product quality and safety and to better understand aspects related to factors influencing flavour composition and product deterioration, and to determine how these relate to both consumer satisfaction, health and shelf life (Szafnauer & Hughes, 2020). Therefore, the identification of effective methods to detect, monitor and control lipid oxidation in packaged meat and meat products is of great interest (Amaral et al., 2018b).

The primary products formed from autoxidation are hydroperoxides, which are very unstable and breakdown to a range of secondary volatile and semi-volatile oxidation compounds, some of which directly influence sensory perception. These main components of secondary oxidation are aldehydes, ketones and alcohols, and are responsible for lipid oxidation off-flavours and off-odours in meat (Cheng, 2016). Many different procedures exist for monitoring lipid oxidation in meat and meat products with peroxide values or TBARS the most widely used (Rahman et al., 2015). Peroxide values measure hydroperoxides, which are indicative of the early stages of lipid oxidation, and therefore this approach has some limitations in that it can underestimate the extent of lipid oxidation (Domínguez et al., 2019). TBARS or malondialdehyde analysis (MDA) is also widely used but the agent required, 2-thiobarbituric acid can react with interfering compounds resulting in an over estimation of lipid oxidation, and also has a high coefficient of variation (Reitznerová et al., 2017). Methods that identify the specific VOC directly influencing sensory perception can provide additional beneficial information. For example, it is possible to determine the identity and the concentration of the volatile compounds involved, and potentially at what point they begin to adversely impact on sensory perception.

Thus, such approaches may also provide estimates of shelf life, but also provide information that could be used to undertake processing changes that limit their formation or even increase shelf life. This is typically achieved using gas chromatographic approaches, where a suitable extraction technique is followed by separation and quantification by gas chromatography using flame ionization or mass spectrometry detection. The initial extraction element is very important as many of these VOC are thermally sensitive. Some of these gas chromatographic methods have been discussed previously outlining their pro's and con's (Barriuso et al., 2013; Ross & Smith, 2006).

Recently a novel semi-automatic extraction technique has been developed called high-capacity sorptive (HiSorb) extraction (Markes International Ltd., Bridgend, UK)**,** which is coupled to a thermal desorption (TD) gas chromatography-mass spectrometry (GC-MS) system. HiSorb provides a versatile and highly sensitive alternative to traditional volatile sample preparation methods, such as solvent extraction, solid-phase micro-extraction (SPME), or static headspace (HS), and involves minimal manual sample handling (Barden & Kelly, 2018). In addition, HiSorb limits of detection are lower than SPME due to a larger capacity sorbent (~65 µl compared to ~0.5 µl for SPME) and also utilises a cryogen-free trap-based pre-concentration step associated with the TD element that increases sensitivity (Cheng et al., 2021). In this study, HiSorb probes with polydimethylsiloxane (PDMS) were evaluated, which were recently shown to be very effective in extracting VOC in whole milk powder in comparison to HS-SPME and TD (Cheng et al., 2021).

The aim of this study was therefore to develop, optimise and validate a HiSorb TD GC-MS to quantify target LOC, and using an untargeted approach to profile VOC in raw beef patties in MAP stored under refrigeration conditions for 21 days.

5.3 Material and methods

5.3.1. Chemicals

All standards (hexanal, heptanal, octanal, nonanal, decanal, furfural, 2-pentanone, 2-heptanone, 1-hexanol, 4-methyl-2-pentanol and 2-methyl-3-heptanone) were supplied by Merck Ireland (Arklow, Co. Wicklow, Ireland) and stored at room temperature. Methanol was also purchased from Merck Ireland (Arklow, Co. Wicklow, Ireland).

5.3.2. Meat samples

Fresh beef (85 % visible lean) was purchased from Ballyburden Meat Processors (Cork, Co. Cork, Ireland) and minced twice through a plate with 4 mm holes (Model P114L, Talsa, Valencia, Spain). The meat was stored at 4 °C until required for analysis and opened prior to the end of use date. Minced beef was formed into 100 g patties using a meat former (Ministeak Burger maker, O.L. Smith Co. Ltd., Italy). The patties were placed in low oxygen permeable (<1 $cm^3/m^2/24$ h at standard temperature and pressure (STP)) polystyrene/ethyl vinyl alcohol/polyethylene trays, using MAP technology. The trays were flushed with 80 % O_2: 20 % CO_2 using a vacuum-sealing unit (VS 100, Gustav Müller and Co. KG, Bad Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH and Co. KG, Witten, Germany). Trays were covered and heat-sealed using a low oxygen permeable (3 $cm^3/m^2/24$ h at STP) laminated barrier film with a polyolefin heat-sealable layer and subsequently stored for up to 21 days at 4 °C under illuminated conditions (616 lux), and analysed at days 0, 3, 7, 10, 14, 17 and 21 of storage. Trays were prepared in duplicate for each day of storage and contained one patty each (n=7 samples, n=14 trays). The gas atmosphere (O_2 % and CO_2 %) in the packs was checked on each sampling day during the packaging trial using a gas analyser (CheckMate 9900, PBI-DanSensor, Denmark) where the instrument needle was inserted through a rubber septum attached to the lidding material.

5.3.3. Proximate analysis

A CEM SMART and a SMART Trac system (CEM GmbH, Kamp-Lintfort, Germany) were used to measure the moisture and fat content, respectively, of fresh beef patties (Leffler et al., 2008).

5.3.4. Colour measurement

The surface colour of the beef patties was measured using a Konica Minolta CR-400 Chroma Meter (Minolta Camera Co., Osaka, Japan). Six readings were taken per storage time and averaged for statistical analysis. Samples were removed from the MAP and allowed to bloom for 20 min prior to evaluating colour parameters. The Chroma-Meter consisted of a measuring head (CR-400), with an 8 mm diameter measuring area, a 2° standard observer, and a data processor (DP-400). The Chroma Meter was calibrated on the CIE LAB colour space system using a white tile (L^* = 94.31, a^* = -0.45, b^* = 3.90). The 'L^*' value represents lightness (L^*=0 yields black and L^*=100 indicates diffuse white) and 'a^*' and 'b^*' values represent redness and yellowness, respectively (a^*, negative values indicate green while positive values indicate magenta and b^*, negative values indicate blue and positive values indicate yellow). Delta E 2000 formula was used to measure the visible difference between two colours, in this case between samples from day 0 and days 3, 7, 10, 14, 17, 21 of storage. ColorTools Excel add-in was used to apply the formula. A smaller Delta E value (ΔE*) represents a closer colour match.

5.3.5. Microbiological analysis

The beef patties were tested for *enterobacteriaceae* (ISO 21528-2: 2017), coliforms (ISO 4832: 2016), *pseudomonas* spp (ISO 13720: 2010), aerobic colony count (ISO 4833-1: 2013), ß-glucuronidase + *E. coli* (ISO 16649-2: 2001), and mesophilic lactic acid bacteria (LAB) (ISO 15214: 1998). Microbiological analysis of fresh beef patties was carried out on days 3, 7, 10, 14, 17 and 21 of storage. The

results were expressed as logarithms of colony-forming units per gram of sample (log CFU/g).

5.3.6. TBARS

Lipid oxidation was measured using the 2-thiobarbituric acid (TBA) assay as described by Siu and Draper (1978) (Siu and Draper, 1978). Chopped beef samples (5 g) were homogenised for 1.5 min in 25 ml distilled water using an Ultra Turrax tissue homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen, Germany). Trichloroacetic acid (TCA) 10 % was added (25 ml) and the mixture was shaken vigorously and filtered through Whatman No. 1 filter paper. In screw-capped test tubes, 4 ml of clear filtrate was added to 1 ml of 0.06 M TBA. The tubes were placed in a water bath and held at 80 °C for 90 min. The absorbance of the filtrate was measured spectrophotometrically (Cary 300 Bio, UV-vis spectrophotometer, Varian Instruments, CA, USA) at 532 nm against a blank containing all reagents (2 ml distilled water, 2 ml 10 % TCA and 1 ml of 0.06 M TBA reagent). The MDA content of the samples was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Results were expressed as TBARS in mg MDA/kg beef.

5.3.7. HiSorb TD GC-MS

5.3.7.1. Standard solution preparation

We selected nine target LOC for quantification: hexanal, heptanal, octanal, nonanal, decanal, furfural, 2-pentanone, 2-heptanone and 1-hexanol. Selection was based on available literature regarding lipid oxidation in raw beef (Casaburi et al., 2015; Gorraiz et al., 2002; Insausti et al., 2002; Pérez et al., 2008; Saraiva et al., 2015; Schindler et al., 2010). 4-Methyl-2-pentanol and 2-methyl-3-heptanone were selected as internal standards.

All standards were prepared at 1000 mg/kg in methanol and stored at -18 °C until required, but never longer than 3 months. Prior to analysis, solutions were

sonicated using a Decon FSI100b ultrasonic bath (Decon Ultrasonics Ltd., UK) at room temperature for 10 min to ensure all the compounds were dissolved. For all calibration curves and when preparing standards for accuracy and precision studies the standards were diluted to the necessary concentrations using distilled water.

5.3.7.2. Sample preparation

Beef patties were removed from refrigeration (4 °C) and allowed to adjust to room temperature for 20 min. The patties were then cut into smaller pieces, weighed and placed into 20 ml clear glass headspace vials with crimp seals/top and prefitted PTFE septa (C-HSCSC-20C-50) (Markes International Ltd., Bridgend, UK). One hundred µl of internal standard (IS) (5µg/g sample) mixture was added to every sample. An easy grip manual crimper for aluminium caps was used to seal the vials (Restek Corporation, Bellefonte, PA, USA). Each sample was prepared in triplicate (n = 3) for volatile extraction. PDMS HiSorb probes (product Code: H1-AXAAC) were inserted into the headspace vials. These were then placed in a HiSorb Agitator (U-HSAG-20) (Markes International Ltd., Bridgend, UK) where volatiles were extracted under controlled conditions as described subsequently. Once sampling was complete, the HiSorb probes were removed from the sample vial, rinsed with distilled water to remove any residue from the surface, dried with a lint-free tissue and inserted into a capped TD tube prior to TD GC-MS analysis.

5.3.7.3. TD GC-MS analysis

A Unity 2 Thermal Desorption Unit (Markes International Ltd., Bridgend, UK), connected to a Series 2 Ultra TD autosampler (Markes International Ltd., Bridgend, UK) was used to concentrate the VOC and remove any excess moisture prior to direct transfer to the GC-MS. The Unity 2 was operated using nitrogen as a purge gas set at 50 psi. Helium carrier gas was used and set at a constant flow rate of 1.2 ml/min. The TD tubes were dry purged for 2 min using a 1:20 split. A two-stage desorption was employed: the first stage desorption was performed at 150 °C and held for 5 min, the

second stage desorption was performed at 300 °C and held for 5 min. A 1:10 split was used for final tube desorption. The cold trap used was a material emissions focussing trap (U-T12ME-2S) held at 30 °C during tube desorption with a gas flow of 50 ml/min. Prior to trap desorption, a 2 min pre-trap fire purge was performed with a 1:50 split. Trap desorption was performed at 30-300 °C at a rate of 24 °C/min and held for 5 min with 1:10 split.

The GC-MS system was an Agilent 7890A GC with an Agilent 5977B mass spectrometry detector (MSD) (Agilent Technologies Ltd., Cork, Ireland). The column used was a capillary DB-624 UI (60 m x 0.3 mm x 1.8 µm) (Agilent Technologies Ltd., Ireland) with helium as the carrier gas. The column temperature started at 40 °C held for 5 min, increased to 230 °C at 5 °C/min and held at 230 °C for 35 min. The total run time was 78 min. Injector temperature was set at 250 °C. The mass spectra of VOC were generated by a MS quadrupole detector with ionisation voltage of 70 eV, 3.32 scans/s and a scanning mass range of 35-350 amu. The ion source temperature was 220 °C and the interface temperature was set at 280 °C. Autotunes were carried out weekly.

VOC identification was performed by comparing their mass spectra to the NIST 2014 mass spectral library and an in-house library created using authentic external compounds using MassHunter Qualitative Analysis Software v B.08.00 (Agilent Technologies, Palo Alto, CA, USA). Spectral deconvolution was also performed to aid in the identification of co-eluting compounds using Automatic Mass Spectral Deconvolution and Identification System (AMDIS) (http://chemdata.nist.gov/mass-spc/amdis/). Linear retention indices (LRI) were determined using the method by Van Den Dool and Kratz (van Den Dool and Dec. Kratz, 1963) and matched against peer reviewed publications (Corral et al., 2015; Gallego et al., 2012; Gianelli et al., 2011). Positive identification was only attained once the mass spectral match reached >80 %, using external standards where possible and when LRI were within 10 % of referenced values. The results were expressed in abundance values.

5.3.7.4. Optimisation of HiSorb volatile extraction conditions

Response surface methodology (RSM) is a widely used systematic design for process development and optimization (Myers et al., 2004). RSM was used to assess the effects of extraction conditions by HiSorb on the abundance of the nine targeted LOC in the raw beef patties. A three-factor central composite rotatable design (CCRD) (2^3) was employed to determine the optimum extraction conditions that result in the greatest total area value for each target compound in the beef patties. Literature suggests that extraction temperature (T) (George et al., 2018) and extraction time (t) (Kim, 2017) have the most significant effect on extraction efficiency of VOC from food matrices. Therefore, optimization of the extraction parameters was carried out using the following independent variables, sample amount (g), extraction time (min) and extraction temperature (°C). Sample amount ranged from 1 to 5 g, extraction time from 60 to 180 min, and extraction temperature from 40 to 72 °C (Table 5.1). A total of 20 experiments were generated using the Design Expert® software (Version 12, Stat. Ease Inc., Minneapolis, USA) (Table 5.1). Experiments were run randomly to minimize the effects of unexplained variability in the actual responses. Experiments with three independent variables consisting of eight factorial, six center and six axial points were carried out (Table 5.1).

Table 5.1. Central composite design for the HiSorb extraction of LOC in raw beef patties planned using Design Expert®. Independent variables were sample amount (x_1, S), extraction time (x_2, t) and extraction temperature (x_3, T).

Run	Sample amount (g, x_1)	Extraction time (min, x_2)	Extraction temperature (°C, x_3)	Space type	Area response[a] (E+07)
1	3	120	56	Center	2.73
2	5	120	56	Axial	2.33
3	3	180	56	Axial	3.31
4	3	120	56	Center	2.64
5	1	120	56	Axial	2.87
6	5	60	72	Factorial	1.92
7	3	120	56	Center	2.62
8	1	180	72	Factorial	3.34
9	3	120	72	Axial	2.76
10	5	60	40	Factorial	1.78
11	3	120	56	Center	2.21
12	5	180	72	Factorial	3.73
13	3	120	40	Axial	2.09
14	1	60	40	Factorial	2.75
15	5	180	40	Factorial	3.59
16	3	60	56	Axial	1.47
17	1	60	72	Factorial	2.30
18	3	120	56	Center	2.09
19	1	180	40	Factorial	4.16
20	3	120	56	Center	2.57

[a] The area response corresponds to the total area for the targeted LOC.

5.3.7.5. Calibration, linearity, limits of detection, limits of quantification and precision

When constructing calibration curves or performing accuracy and precision studies, a set of beef patty (1 g) was each spiked with 100 µl of the standard mixture. These measurements were compared against an unspiked meat sample where the responses of each standard was calculated from the difference between the spiked and unspiked results. Samples were prepared in triplicate for HiSorb extraction (n = 3).

Calibration curves for the selected targeted LOC were created spiking several beef patty samples (containing IS) at optimized extraction conditions with increasing concentrations of the standard mixture. Target and qualifier ions for each compound were established and two 5-point calibration curves were constructed with a concentration range of 100-1000 µg/kg sample for 2-pentanone, 2-heptanone and 1-hexanol, and 1000-5000 µg/kg sample for hexanal, heptanal, octanal, nonanal, decanal, and furfural (given that concentration of aldehydes was higher in the samples). Calibration curves were established based on the correction factors of the compounds quantifier ions against each internal standard (4-methyl-2-pentanol was used as the internal standard for the quantification of aldehydes and alcohols, and 2-methyl-3-heptanone was used for the quantification of ketones). Quantification was performed on Masshunter Quantitative Analysis software v B.09.00 (Agilent Technologies, Palo Alto, CA, USA). Measurements for each individual LOC were calculated from the difference between the spiked and an unspiked beef patties (where only internal standard was added). Results were expressed as µg/kg sample.

Linearity was determined using two standard calibration curves at 10 concentration levels, 100-1000 µg/kg for 2-pentanone, 2-heptanone and 1-hexanol; and 1000-5000 µg/kg for hexanal, heptanal, octanal, nonanal, decanal and furfural. In addition, regression curves (ratio of the standard quantifier ion peak areas to the relevant internal standard quantifier ion peak area against the concentration) were evaluated and results were expressed by the squared determination coefficient (R^2).

Limits of detection (LOD) and limits of quantification (LOQ) were determined using the linear regression analysis (Evard et al., 2016). Calibration curves, at concentrations of 100, 300, 500, 700 and 1000 µg/kg (n = 3 each) and 1000, 2000, 3000, 4000 and 5000 µg/kg (n = 3 each), were obtained. The LOD levels were calculated as 3.3 x S_y/b, where b is the slope and S_y is the standard deviation of the y-intercepts of linear regression lines. The LOQ were obtained from a standard curve where the lowest point on the curve yielding reproducible results was accepted as the LOQ for each compound.

Precision was established by assessing the repeatability of the standard mixture (hexanal, heptanal, octanal, nonanal, decanal, furfural, 2-pentanone, 2-heptanone and 1-hexanol) at 1 µg/g sample. Repeatability is expressed as the percent relative standard deviation (% RSD) of relevant peak areas of a number of replicates (% RSD = (standard deviation (SD)/mean)*100). The intraday repeatability was estimated by analyzing 3 replicates (n = 3) of samples in a day. The interday precision was determined by the analysis of 21 replicates (n = 21) of samples over the course of 7 days.

5.3.8. Data analysis

Colour changes and changes in LOC measurements were evaluated by Bonferroni multiple comparison analysis (one-way ANOVA), using statistical package for the social sciences (SPSS) software version 24 (IBM Corp., Armonk, NY), to determine if there were statistically significant differences ($P < 0.05$) across time. Correlations between TBARS values and LOC were determined by correlation analyses using the Pearson's linear correlation coefficient with the latter statistical software package. R software (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) for statistical computing and graphics was used to analyse the untargeted VOC profile of the samples and for the generation of principal component analysis (PCA) and heatmap plots. The R package metaMS (Wehrens et al., 2014) was used to batch process chromatograms for untargeted VOC profiling.

The packages FactoMineR (Lê et al., 2008) and Factoextra (Kassambara and Mundt, 2020) were used to perform PCA and generate plots. Hierarchical clustering heatmaps were generated using the R package pheatmap (Kolde, 2019).

5.4 Results and discussion

5.4.1. Optimization of the volatile HS-HiSorb extraction

RSM evaluated the relative significance of variables that influence the process. The optimum conditions for the maximum extraction yield led to an extraction of 1 g of sample agitated for 180 min at a fixed temperature of 40 °C to maintain the samples raw (Fig. 5.1). The resulting R^2 value for the model was 0.9074, which according to the F test, demonstrated significant regression, that is, good correlation between the experimental data and the fitted model. The lack of fit F-value of 1.50 implied the lack of fit is not significant relative to the pure error. The statistical analysis (one-way ANOVA) determined as significant for the overall model (P = 0.0004), extraction time (P <0.001) and sample amount (P = 0.0478). The adjusted R^2 and predicted R^2 were 0.824 and 0.529, respectively.

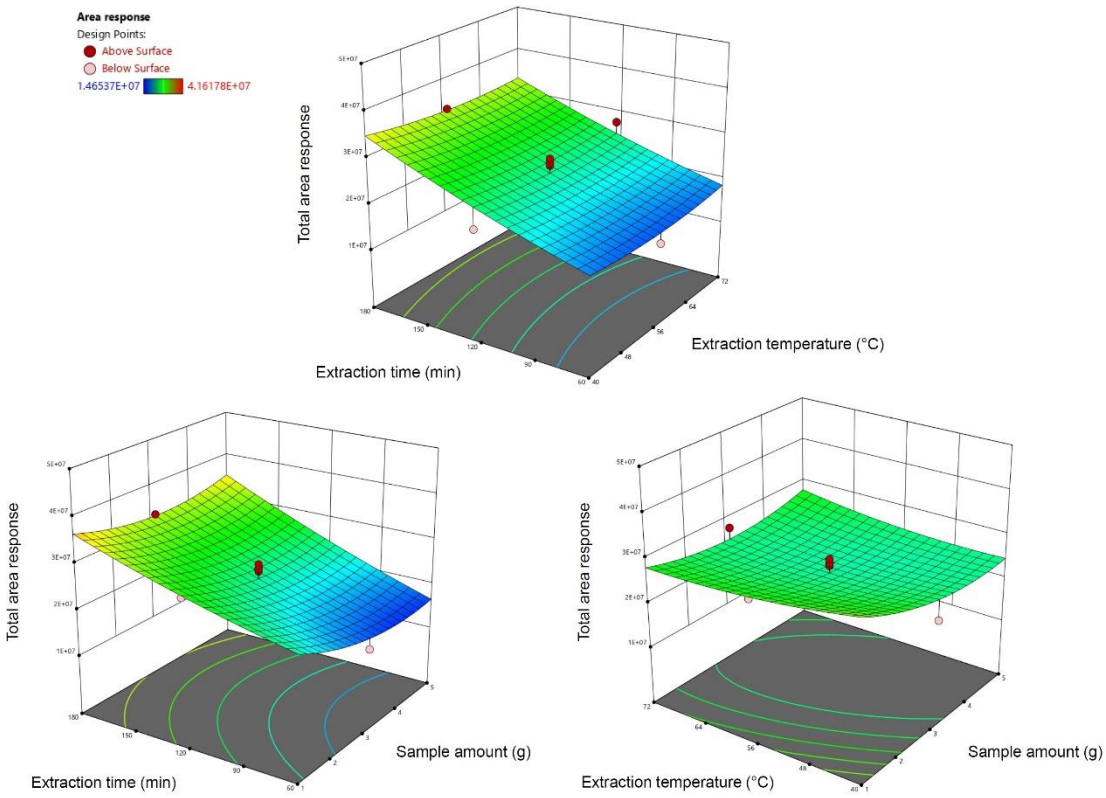


Figure 5.1. Response surface graphs for the dependent variable (total area value) vs. sample amount (g), extraction time (min) and extraction temperature (°C) showing the optimum extraction conditions for the target compounds from beef patties.

5.4.2. Validation of the volatile HS-HiSorb extraction method

The calibration curve equations, linear range, the determination coefficient (R^2), LOD, LOQ, and precision data for the LOC can be found in Table 5.2. Linearity was obtained over the linear concentration range of 100-1000 µg/kg for the ketones (2-pentanone and 2-heptanone) and 1-hexanol, and 1000-5000 µg/kg for the aldehydes (heptanal, octanal, nonanal, decanal and furfural). Correlation coefficients of ≥0.962 were achieved for ketones and 0.988 for 1-hexanol, whereas the aldehydes demonstrated poorer linearity with an R^2 between 0.958 to 0.998 (Table 5.2). We were not able to obtain a good calibration curve for hexanal at any concentration range due to coelution with an unidentified compound.

LOD and LOQ were evaluated based on linear regression lines. LOD varied between 100-~1050 µg/kg for all target LOC, lowest for 1-hexanol and highest for

decanal. LOQ were lowest for the ketones and 1-hexanol (~300 - 550 µg/kg), but significantly higher for the aldehydes 960 - ~3200 µg/kg, except for hexanal which as mentioned co-eluted with an unknown VOC resulting in a much higher LOD (757 µg/kg) (Table 5.2).

Precision was monitored over the course of the analysis by analyzing the standard mixture at 5 µg/g in the samples. Intraday repeatability was established by analysing 3 samples (*n*= 3) in 1 day with 0.6 to 3.7 % RSD achieved across all compounds. The interday precision was monitored in 21 samples (n = 21) for 7 days. Good repeatability, 3.3 to 9.3 % RSD was achieved across most compounds, except for 2-pentanone and furfural, which had higher % RSD, at 11.3 and 14.4 %, respectively (Table 5.2).

Overall, the method demonstrated a good linear range, correlation coefficient, LOD, LOQ and intraday and interday for all targeted LOC, but was least effective for hexanal due to coelution.

Table 5.2. Validation data for lipid oxidation compounds, with linear calibration, LOD, LOQ, and precision results.

LOC	Line equation	Linear range (µg/kg)	Coefficient of determination (R^2)	LOD (µg/kg)	LOQ (µg/kg)	Intraday RSD (%)[a]	Interday RSD (%)[b]
Hexanal	y = 0.523x - 0.0325	1000-5000	0.5931	757	2293	1.1	1.3
Heptanal	y = 0.1915x - 0.0119	1000-5000	0.9659	938	2843	1.0	4.6
Octanal	y = 0.2259x - 0.0126	1000-5000	0.9687	898	2722	1.0	7.1
Nonanal	y = 0.3265x - 0.0029	1000-5000	0.9980	317	960	0.6	3.6
Decanal	y = 0.1818x - 0.0103	1000-5000	0.9579	1048	3175	2.2	9.3
Furfural	y = 0.0965x - 0.0076	1000-5000	0.9685	901	2731	3.7	14.4
1-Hexanol	y = 0.7653x - 0.004	100-1000	0.9879	101	306	2.7	4.2
2-Pentanone	y = 0.7548x + 0.0188	100-1000	0.9620	182	551	3.5	11.3
2-Heptanone	y = 1.2637x + 0.0163	100-1000	0.9853	112	338	3.4	3.3

LOC, lipid oxidation compound; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviation; [a] Average values obtained from 3 samples (n = 3) analysed in a single day; [b] Average values obtained from 21 samples (n = 21) analysed over 7 days.

5.4.3. Evaluation of beef patties over storage

5.4.3.1. Gas and proximate analysis

At the beginning of the trial, beef patties in MAP trays contained 71.5 % O_2 and 21.3 % CO_2 and at the end of the trial 63.0 % O_2 and 30.7 % CO_2.

The moisture and fat content of fresh beef patties was 59.9 ± 4.1 and 21.5 ± 6.0 %, respectively. The moisture content was expected to be inversely associated with fat content, higher fat (%) results in lower moisture. This assumption is supported by findings in Garicano Vilar et al. (Garicano-Vilar et al., 2020) and Tobin et al. (Tobin et al., 2012).

5.4.3.2. Colour measurement

Colour perception plays a major role in the evaluation of meat quality as consumers use colour as an indicator of freshness as it strongly influences consumer purchasing decisions (Lanari et al., 2002). Package oxygen content 20 % or higher is beneficial for colour stability (Jakobsen & Bertelsen, 2000). Significant colour differences (P <0.002) were seen between day 0 and days 3-21 of storage (Table 5.3). L^* values (lightness) were found to be significantly higher (P = 0.002) as time passed. a^* values (redness), the most important colour parameter for fresh meat (Olivera et al., 2013), decreased significantly (P <0.001) with increasing storage time, specifically after day 7. The b^* values (blue/yellow) were also significantly lower (P <0.001) in the samples (more blue, less yellow), but this value fluctuated over storage time. Total colour change (ΔE*) was calculated to study the differences between raw beef patties in relation to storage time. The reference taken in each case was the colour of day 0 patties. The value of ΔE increased as storage time increased (Table 5.3). Two colours can be optically distinguished if $\Delta E \geq 1$ (Dattner & Bohn, 2015) and, according to Francis and Clydesdale (Francis & Clydesdale, 1977), when ΔE is >3 colour differences are obvious. In this sense, from day 3 of storage onwards the samples were perceived as having a significant colour deviation from day 0 (Table 5.3). All colour measurements were also significantly affected by storage time in the study by Ijaz et al. (Ijaz et al., 2020), where dark firm dry beef was stored at 4 °C for 7 days and colour changes were perceived from day 1 to day 3. Similar results were also observed by Olivera et al. (Olivera et al., 2013) in beef.

Table 5.3. Colour changes of raw beef patties throughout storage time under MAP.

Storage	Day 0	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21	
	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	*P*-value
L*	49.91 ± 0.40 ^{ab}	45.24 ± 0.74 ^{ac}	54.11 ± 0.34 ^{bd}	52.39 ± 0.05 ^{ad}	54.17 ± 1.85 ^{bd}	55.96 ± 1.36 ^{bd}	57.78 ± 1.71 ^{de}	0.002
a*	28.44 ± 0.73 ^{a}	21.02 ± 1.06 ^{b}	7.13 ± 0.01 ^{c}	6.76 ± 0.15 ^{c}	5.78 ± 0.10 ^{c}	5.72 ± 0.15 ^{c}	4.86 ± 0.40 ^{c}	<0.001
b*	16.90 ± 0.83 ^{a}	12.81 ± 0.21 ^{bc}	12.11 ± 0.08 ^{b}	12.72 ± 0.21 ^{bd}	13.61 ± 0.14 ^{bd}	14.24 ± 0.18 ^{bd}	14.58 ± 0.14 ^{cd}	<0.001
ΔE*	N/A	5.95	14.50	14.60	16.17	16.88	18.44	N/A

M, mean; SE, standard error; N/A, not applicable; *P* <0.05 considered statistically significant; $^{a–e}$, means in the same row bearing different superscript letter denote statistically significant differences (*P* <0.05).

5.4.3.3. Microbiological analysis

Monitoring and evaluation of the microbiological contamination of beef is pertinent for its safety and quality. Meat has been found to be a prime vehicle for the dissemination of foodborne pathogens to humans (Nychas et al., 2008). Water content activity (aW) of fresh meat and its neutral pH play a vital role in the growth of microbes (Nychas et al., 2008). Thus, it was considered pertinent to undertake microbiological analysis of these samples (Table 5.4) in order to discard any potential changes in the samples caused by microbes rather than from lipid oxidation.

Table 5.4. Microbial counts (CFU/g) of beef patties across storage time.

	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21
Test	CFU/g	CFU/g	CFU/g	CFU/g	CFU/g	CFU/g
Enterobacteriaceae	110000	>150000	78000	>150000	88000	35000
Coliforms	>150000	>150000	18000	120000	86000	50000
***Pseudomonas* spp**	>150000	<100	<100	<100	>150000	>90000
Aerobic colony count	>30000000	>30000000	>30000000	>30000000	>30000000	>30000000
ß-Glucuronidase + *E. coli*	60	60	20	30	10	40
Mesophilic LAB	>3000000	310000	>3000000	>3000000	>3000000	>3000000

CFU, colony-forming unit; LAB, lactic acid bacteria.

According to the Health Protection Agency guidelines for assessing microbiological safety of ready-to-eat foods (Health Protection Agency, 2009), the mean aerobic colony count levels (an indicator of quality, not safety) were within borderline quality (10^6 - $<10^8$ CFU/g) throughout storage (Table 5.4). In meat products, the flora frequently consists almost entirely of LAB (mainly lactobacilli and streptococci), which can grow well at refrigeration temperatures. Spoilage will eventually occur at a level of around 10^9 CFU/g due to the production of lactic acid. Levels of LAB remained below spoilage threshold in our study (Table 5.4); whereas in Jääskeläine et al. (Jääskeläinen et al., 2016) LAB reached the maximum cell contents on day 9 in high oxygen MAP beef. Spoilage caused by gram-negative bacteria, such as pseudomonads, is likely noticeable at 10^7-10^8 CFU/g; as they tend to produce taints, discolouration, and slime (Jääskeläinen et al., 2016). *Pseudomonas* spp levels remained below these levels for the duration of storage (Table 5.4). Although *E. coli* should not be detected in ready-to-eat foods, low levels may occasionally be found

in raw meat (Health Protection Agency, 2009) as in this study. *Enterobacteriaceae* remained below 6 logs in this study (Table 5.4), and can occur in the environment as well as the gut (Jääskeläinen et al., 2016). According to the Food Safety Authority of Ireland Criteria (Food Safety Authority of Ireland, 2020) >100 CFU/g of faecal coliforms in minced beef is unsatisfactory. Therefore, using the latter criteria, all our beef samples exceeded the microbiological criterion based on faecal coliform counts, but were not unsafe (Table 5.4).

5.4.3.4. Lipid oxidation measurement (2-Thiobarbituric acid analysis)

Minced meat and meat products undergo oxidative changes more quickly as grinding exposes lipid membranes to metal oxidation catalysts (Lee et al., 2011). Figure 5.2 shows the effect of time on TBARS values of raw beef patties during 21 days of storage. Initial TBARS level (day 0) was 0.66 ± 0.09 mg MDA/kg. The TBARS levels continued to increase, ranging from 0.66 to 7.21 mg MDA/kg from the beginning to day 17 of storage. At day 21, a slight drop on the TBARS value was observed (7.01 ± 0.64 mg MDA/kg), however in general TBARS content increased throughout the experiment (P <0.001), in agreement with previous studies (Faustman et al., 2010; Quevedo et al., 2013; Wilkinson et al., 2001). Significant differences were observed between day 0 and days 7, 10, 14, 17 and 21 (P <0.02). No statistically significant differences were observed between day 0 and day 3 (P >0.05), day 3 and day 7 (P = 0.206) and day 17 and day 21 (P >0.05).

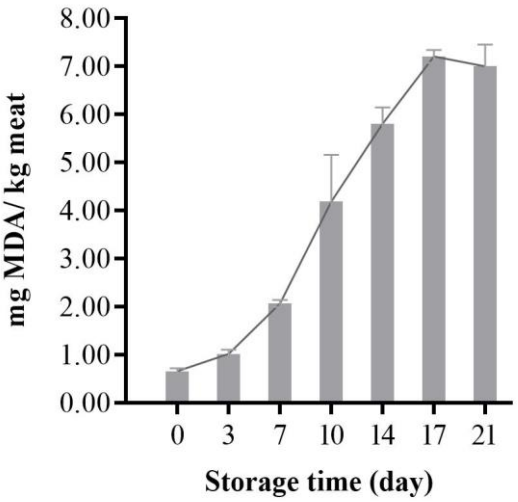


Figure 5.2. Lipid oxidation of beef patties during storage measured by TBARS (mg MDA/kg).

Some previous studies have suggested TBARS limits in beef for consumer acceptance. McKenna et al. (McKenna et al., 2005) considered 1.0 mg MDA/kg as the limiting threshold of TBARS values for beef steaks. On the contrary, Campo et al. (Campo et al., 2006), considered ~2.0 mg MDA/kg as the limiting threshold for the acceptability of oxidised beef; while, Hughes et al. (Hughes et al., 2015) found TBARS levels between 2.6 and 3.1 mg MDA/kg in beef striploins were still acceptable to consumers. These inconsistent outcomes are noteworthy since, this method has been proposed to be the most suitable method for determining lipid oxidation level in meat due to the absence of a heat treatment step (Estevez et al., 2008). Based on the aforementioned examples, the beef patties in study would not be considered suitable for human consumption beyond day 3 (1.02 ± 0.13 mg MDA/kg) or day 7 of storage (2.07 ± 0.10 mg MDA/kg). However, the acceptance range for beef patties is likely to be different from beef steaks or striploins.

5.4.3.5. Assessment of LOC over storage by HiSorb TD GC-MS

The targeted LOC were quantified over storage in MAP (Table 5.5).

Table 5.5. Concentrations (µg/ kg of beef; n = 3) of the 9 targeted LOC quantified in the beef patties across storage time

LOC	Day 0	Day 3	Day 7	Day 10	Day 14	Day 17	Day 21	
				µg/kg ± SE				*P*-Value
Hexanal	170±16.7^{abc}	147±0.3^{b}	160±9.6^{abc}	223±19.4^{ce}	281±14.3^{de}	284±5.0^{de}	302±7.5^{d}	<0.001*
Heptanal	154±16.7^{abc}	110±1.2 ^{b}	133±3.2^{abc}	146±7.5^{abc}	179±5.6^{ce}	206±1.4^{de}	242±8.5^{d}	<0.001*
Octanal	100±14.4^{ab}	81±0.7^{b}	92±3.4^{ab}	109±6.6^{ab}	106±2.9^{ab}	111±0.5^{ab}	120±3.0^{ac}	0.02*
Nonanal	424±62.8^{a}	219±10.3^{bc}	311±19.0^{ac}	300±21.3^{ac}	277±12.2^{bc}	274±6.8^{bc}	266±3.8^{bc}	0.005*
Decanal	133±12.8^{a}	113±9.1^{a}	121±5.7^{a}	120±3.7^{a}	116±3.3^{a}	112±2.9^{a}	106±1.9^{a}	0.234
Furfural	11±4.1^{a}	14±0.7^{ac}	20±3.3^{ac}	12±2.2^{ac}	23±1.0^{bc}	17±0.9^{ac}	22±1.4^{ac}	0.008*
1-Hexanol	20±0.8^{a}	25±0.7^{a}	33±0.4^{a}	67±8.1^{c}	128±4.9^{b}	133±6.7^{b}	226±3.3^{d}	<0.001*
2-Pentanone	74±3.6^{a}	70±1.6^{a}	30±35.9^{a}	60±9.6^{a}	42±4.5^{a}	48±8.7^{a}	43±13.0^{a}	0.172
2-Heptanone	38±1.6^{a}	36±0.7^{a}	31±4.5^{a}	32±2.9^{a}	28±0.7^{a}	30±1.7^{a}	29±2.0^{a}	0.029*

LOC, lipid oxidation compound; $^{a-e}$, mean values in the same row bearing different superscripts indicate significant difference (P <0.05) between the storage day. *Statistically significant difference (P <0.05)).

Only seven of the targeted LOC (hexanal, heptanal, octanal, nonanal, furfural, 1-hexanol and 2-heptanone) changed significantly over storage (P <0.05) (Table 5.5). All aldehydes (hexanal, heptanal, octanal, nonanal, and decanal) showed a similar pattern, dropping slightly from day 0 to day 3, then increasing thereafter. Both 1-hexanol and 2-heptanone increased from day 0 to 21, while both furfural and 2-pentanone increased from day 0 to 7, but varied thereafter (Table 5.5).

The validated method has the ability to detect and quantify the targeted LOC in beef samples at low concentrations (400-3000 µg/kg), with the exception of hexanal, which, as mentioned co-eluted with an unidentifiable compound. The validation range 100-5000 µg/kg was within the levels of most target compounds found in the beef patties as determined by calibration curves and by the ratio of the

standard peak areas to the relevant internal standard peak area versus the concentration.

The effect of storage time on the targeted LOC is not surprising since they are major lipid oxidation products and the high concentration of oxygen in MAP promotes oxidation. However, a high variability was found as previously reported (Estévez et al., 2003) and may explain the low R^2 generally found throughout storage for the aldehydes in particular. Saturated aldehydes have been previously described as being affected by storage time in raw beef packed in the presence of high concentrations of oxygen (Insausti et al., 2002).

The methyl ketones (2-pentanone and 2-heptanone) that derive from short- and medium-chain fatty acid of less than 14 C-atoms are formed through enzymatic hydrolyzation and β-oxidation of triacylglycerides (Fruehwirth et al., 2021) increased up to day 17. Resconi et al. (Resconi et al., 2018) suggested 2-heptanone as a potential predictor of odour shelf life of beef. It has also been proposed as a candidate for aroma differences of cooked beef at different levels of oxidation (Resconi et al., 2012), and has also been shown to increase incrementally with storage time in raw meat (Estévez et al., 2003; Insausti et al., 2002). Olaoye (2016) (Olaoye, 2016) found that 2-pentanone increased in *Balangu* meat during 3 days-storage; similarly, Mottram (Mottram, 1998) observed an increase in ketone content (i.e. 2-mercapto-2-pentanone, 3-mercapto-2-pentanone) with increased lipid oxidation.

1-Hexanol increased over storage in this study and, although mainly derived from the oxidation of linoleic acid, it may also be due to increases in the concentration of free amino acids and sugars, as alcohols are also formed when amino acids and ribose interact in the lipid oxidation pathway (Elmore et al., 2002). Some studies have found that amino acids and ribose increased in meat over storage (Koutsidis et al., 2008); however, other studies have not found an increase of 1-hexanol over storage (Ma et al., 2012; Watanabe et al., 2015). Obviously alcohols can also increase in stored meat, due to the growth of natural spoilage bacteria (Casaburi et al., 2015), therefore, it is essential to monitor this, although not a factor in this study. Overall, 1-hexanol appears to be an excellent biomarker for lipid oxidation, as in this study it has good linear range, correlation coefficient, LOD, LOQ and precision.

A matrix effect was observed in this study as evident by the total ion count of the standards in comparison to the beef patty samples including the same standards (Fig. 5.3 A and B). The response of the targeted VOC in the spiked samples was less than that of the standards at the same concentration, but most pronounced with hexanal and furfural. For example, in Figure 5.3 both hexanal and furfural had well-defined peaks in the standard (Fig. 5.3(A)) but had a reduced response when spiked in the sample Fig. 5.3(B). The matrix effect in this case even though possibly influenced by the chemical structures and/ or physical properties of the analytes of interest, was more likely influenced by the complex matrix and/ or the analyte/ matrix concentration ratio (De Sousa Freitas & Lanças, 2009). Thus, issues we have seen with hexanal in the beef patties may not be present in other beef samples, but this work highlights the potential complexity of these types of studies and that bespoke solutions are often required for individual sample types.

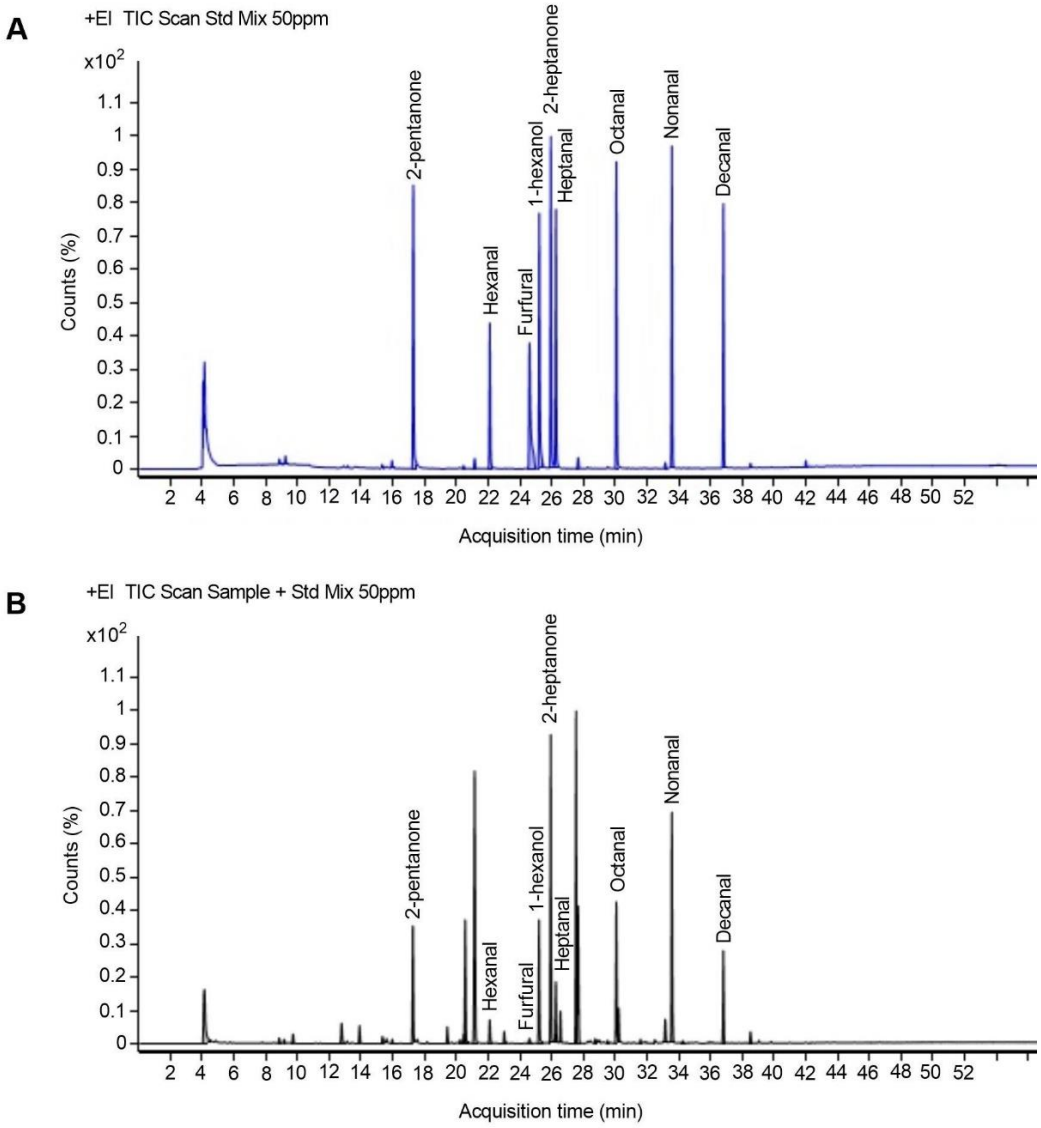


Figure 5.3. (A) Total ion count (TIC) chromatogram obtained from 100 µl of the standard mix prepared at 0.005 % (w/v); (B) TIC chromatogram obtained from a beef patty sample (1 g) spiked with 100 µl of the standard mix prepared at 0.005 % (w/v) plus 100 µl of the internal standard mix prepared at 0.005 % (w/v).

Four of the targeted LOC (1-hexanol, 2-heptanone, heptanal and hexanal) that increased throughout storage were highly correlated with TBARS (r = 0.905; r = 0.793; r = 0.865 and r = 0.976, respectively) (Table 5.6). There are many studies that report a high correlation between TBARS and total aldehydes and hexanal. For example, Domínguez et al. (Domínguez et al., 2014) found a correlation of 0.811 and 0.794, respectively; while Nieto et al. (Nieto et al., 2011) found a correlation between TBARS

and hexanal of 0.936, and Brunton et al. (Brunton et al., 2000) of 0.99. In addition, Resconi et al. (Resconi et al., 2018) reflected the Pearson correlations between VOC of raw meat in high oxygen MAP and TBARS, concluding that pentanoic, hexanoic and heptanoic acids, 1-hexanol, (E)-2-undecenal, ethyl octanoate, 2-heptanone and 2-pentylfuran were highly correlated with TBARS.

Table 5.6. Pearson correlations coefficients (r) between the LOC and TBARS of beef patties stored in MAP for 21 days.

	TBARS
Hexanal	0.976**
Heptanal	0.865*
Octanal	0.563
Nonanal	-0.394
Decanal	-0.698
Furfural	0.566
2-pentanone	0.409
2-heptanone	0.793*
1-hexanol	0.905**
Days of storage	0.973**

*, correlation is significant at the 0.05 level; **, correlation is significant at the 0.01 level.

5.4.3.6. Assessment of VOC over storage by HiSorb TD GC-MS

Fourteen aldehydes, 9 ketones, 9 alcohols, 7 esters, 4 acids, 2 furans, 2 benzenes, 1 sulphur compound and 1 pyrrole (n = 49 VOC) were identified in these beef patties, which is somewhat similar to the study by Wang et al. (Wang et al., 2018), who detected 39 VOC in raw beef (including hydrocarbons, aldehydes, ketones, alcohols, esters, acids, furan and S-containing compounds). The abundance of individual VOC in this study were quite diverse. Acids, aldehydes, benzenes, esters,

furans and ketones attained their highest levels after 14 days of storage. Alcohols reached their highest levels on day 17 of storage, while the sulphur compound and the pyrrole were only present on days 0 and 3.

The relationship between the individual VOC in these samples over storage is shown in Figure 5.4. The first two principal components of the PCA explained 73 % of the variance (Fig. 5.4). A clear discrimination exists between these samples based on storage time (Fig. 5.4), as evident by the increases in association with more VOC over time. Day 0 was differentiated from all the other samples, with few strong associations with any VOC on the positive side of PC2 and the negative side of PC1 (Fig. 5.4). Samples at day 3 were in the same quadrant on the positive side of PC2 and the negative side of PC1 as samples at Day 0. Day 3 was closely associated with 4-octanone, isopropyl alcohol (2-propanol), methyl acetate, carbon disulphide, undecanal, pyrrole, pentanal, acetone, butanoic acid and 2-propenal. From these VOC those most associated with lipid oxidation are the primary aldehydes (pentanal and undecanal), the 2-alkenal (2-propenal) and the ketone (4-octanone). At day 7 (also in the same quadrant) a stronger association with butanal, nonanal, decanal, 2-methyl-3-heptanone, toluene, methyl octanoate, 4-methyl-2-pentanol and 2-methylpropanal was evident. Only the primary aldehydes (butanal, nonanal, decanal) within these VOC were the main products associated with lipid oxidation. Day 10 was slightly different to day 7 (but just in the negative side of PC1 and in the negative side of PC2), with a similar association to butanal, 2-methylpropanal, but also to benzene, octanal and 1-octanol, with only octanal and 1-octanol products of lipid oxidation. Day 14 and day 17 (positive side of PC1 and PC2) were quite similar with a strong association to many VOC (methyl isobutyrate (2-methylpropanoate), acetoin, furfural, 2,3-butanedione, 2,3-butanediol, 2-methyl butanoic acid, 2-pentanone, 2-heptanone, hexanal, 1-pentanol, 3-methyl butanal, 1-octen-3-ol, (E)-2-nonenal, methyl hexanoate, 2-ethyl furan, methyl valerate (methyl pentanoate), 3-methyl-1-butanol, 2-pentyl furan, methyl heptanoate, benzaldehyde, 2-octanone and 1-hexanol). Many of these are primary or secondary lipid oxidation products; the aldehydes (hexanal, (E)-2-nonenal, benzaldehyde), the alcohols (1-pentanol, 1-octen-3-ol and 1-hexanol) (Al-Dalali et al., 2022), and the methyl ketones (2-pentanone, 2-heptanone and 2-octanone). In addition, both 2-ethyl furan and 2-

pentyl furan are also likely products of oxidation as these can be formed from 2-alkenals in the presence of iron (Grebenteuch et al., 2021). Many of the other VOC are indicative of microbial metabolism from carbohydrates (acetoin, 2,3-butanedione, 2,3-butanediol), or amino acids (2-methyl butanoic acid, 3-methyl butanal, 3-methyl-1-butanol and benzaldehyde) or from esterase activity (methyl isobutyrate, methyl hexanoate, methyl valerate and methyl heptanoate) (Jääskeläinen et al., 2016), which is an indication of increased microbial activity over this period. Day 21 was not strongly associated with any VOC, the abundance of many VOC was lower at day 21 in comparison to day 17. It is unclear as to why this change in VOC profile occurred at this time point, but it is a phenomenon previously observed in other studies (Han et al., 2020; Ludlow et al., 2021; Zareian et al., 2018) and day 21 was distinctly separate from the other time points on the negative side of PC1 and the positive side of PC2. This also corresponds with the TBARS data.

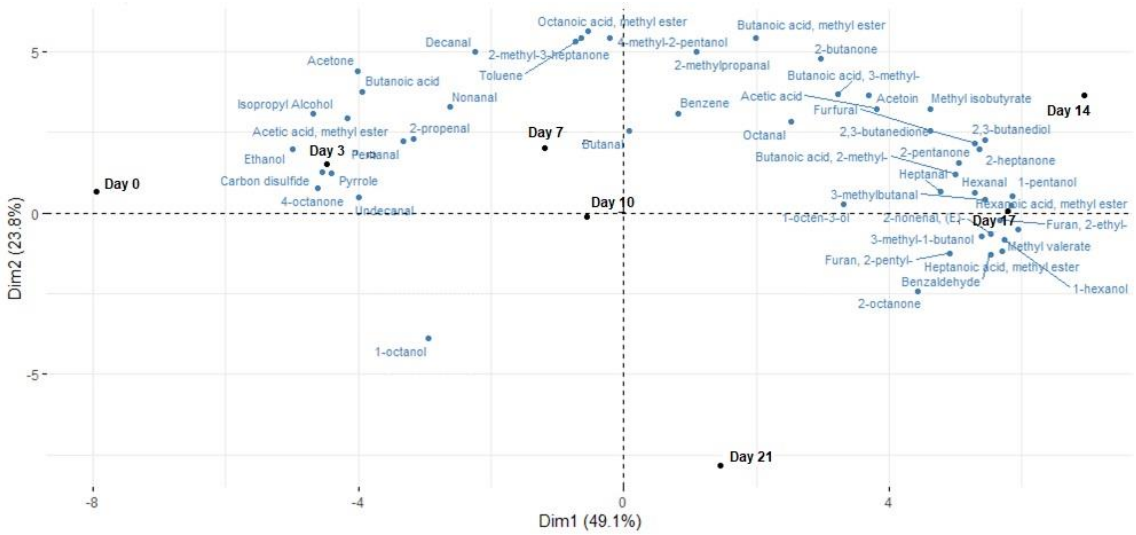


Figure 5.4. Principal component analysis scores plot depicting the distribution of VOC detected by HiSorb TD GC-MS analysis beef patties stored in refrigeration for 21 days in MAP, for the first two principal components (PC1 and PC2).

5.5 Conclusion

This study covers the validation of a novel lipid oxidation method for the quantification of targeted LOC and for profiling untargeted VOC in beef patties. The

overall trend in terms of lipid oxidation by the HiSorb TD GC-MS was similar to TBARS but provides significantly more specific information generated in relation to the targeted LOC, but also the whole VOC profile of the samples over storage. The ability to quantify the main LOC involved at low levels of LOQ in a validated method is beneficial in terms of better understanding factors impacting on lipid oxidation over storage and thus potentially identifying ways to ameliorate shelf life stability. The evolution of specific LOC and VOC from microbial activity over storage was evident**.** In general, the method was most sensitive for both 1-hexanol and 2-heptanone as LOC biomarkers, but was beneficial for all nine LOC investigated. Unfortunately, hexanal, an established key LOC in meat had slightly higher LOD and LOQ due to both matrix effect and co-elution issues.

This study has demonstrated that matrix effects and co-elution can adversely impact the sensitivity of this method for the quantification of specific VOC, and thus simply transferring an optimised VOC GC-MS method for one sample type to another cannot be assumed to provide reliable data unless it has been validated. Further studies are required on more samples to determine the association of both targeted LOC and untargeted VOC in relation to consumer perception in beef patties and other meat products prior to potentially increase shelf life without adversely impacting on product quality**.**

5.6 Limitations

Sorptive extraction works on the principle of absorption, which is an equilibrium process. The maximum achievable extraction efficiency for a particular analyte therefore depends upon its characteristics, and it may not be possible to achieve 100 % recovery in all cases due to the range of factors involved that may influence the process.

5.7 References

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Chapter 6

Chapter 6. Assessing the impact of the inclusion of selected seaweed species on the volatile and odour profile of raw beef patties over refrigerated storage

6.1 Abstract

The incorporation of natural ingredients, such as seaweeds, into processed meat products may have potential as a natural alternative to reduce the rate of lipid oxidation, prevent the development of off-flavour and mitigate colour changes over refrigerated shelf life, all of which adversely impact consumer acceptance. This study analysed the aroma volatile profile, extent of oxidation and colour of raw beef patties reformulated with dried Sea Spaghetti– *Himanthalia elongata* and Nori– *Porphyra umbilicalis*, at 1 and 3 % w/w inclusion levels stored in modified atmosphere packaging (MAP) at 4 °C for 14 days. Higher inclusion rates of seaweed resulted in only slightly lower levels of lipid oxidation with higher total phenolic compounds in Sea Spaghetti than in Nori samples. 2,2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and ferric reducing antioxidant power (FRAP) activities were similar between Sea Spaghetti and Nori samples. Significant colour differences (P <0.05) (L*, a*, b* and ΔE*) were seen over storage, however no significant differences were observed between the experimental and control samples for L*, a* values, with only statistical differences in b* values with higher inclusion rates of seaweed and a greater increase in ΔE* in only the 3 % Sea Spaghetti samples in comparison to the control. The inclusion of seaweed altered the volatile profiles with 82 volatile organic compounds (VOC) in total in the reformulated beef patties and 64 VOC in the control samples, with the volatile profiles changing throughout storage. 78 aroma active volatiles were identified (aldehydes, ketones, acids, alcohols, lactones, esters, furans, alkanes, ethers and phenol) by gas chromatography-olfactometry (GC-O). Odour intensity was impacted by seaweed addition in the order of Nori>Sea Spaghetti>control, however no statistically significant differences (P >0.05) were observed for odour intensity over storage time. Control samples were characterized by 'green, earthy, buttery'

aromas; Sea Spaghetti patties by 'earthy, aldehydic, bready' aromas, and Nori patties by 'aldehydic, cheesy, buttery, chocolate' aromas. The study has demonstrated that significantly more research is required to optimise the inclusion of seaweed species in processed meats in order to enhance shelf life without adversely impacting on key quality parameters.

6.2 Introduction

Lipids, mainly the phospholipid component, play a role in the development of meat flavour and much of that flavour is derived from adipose tissue (Miller, 2014). Additionally, adipose cells serve as reservoirs for storage of aroma compounds derived from external sources (i.e. feedstuffs) that contribute towards flavour-forming reactions (Miller, 2014). However, lipids also contribute to meat flavour through their contribution towards the development of off-flavours, mainly through oxidation (Lee et al., 2020). Lipid oxidation is a complex chain of reactions that occurs in three successive stages (initiation, propagation, and termination). In the termination stage, hydroperoxides radicals react with each other to form compounds such as aldehydes, ketones, alkanes and other hydrocarbons (Domínguez et al., 2019). All these compounds are known to affect the sensory characteristics of meat, being responsible for off-flavour and rancid odour (Kumar et al., 2015), and consequently a unfavourable consumer acceptance (Domínguez et al., 2019; Kumar et al., 2015). Another very important aspect of meat quality and consumer acceptance is meat colour (Gómez and Lorenzo, 2012). If meat colour is not consistent with expectations, consumers will decide not to purchase (Tkacz et al., 2020).

Natural sources of antioxidant micronutrients that are able to oppose the effects of free radicals (Mellouk et al., 2017) and the lipid oxidation process in meat and meat products is of great interest to the industry (Amaral et al., 2018). Over the years, various preservation techniques have been developed to increase food safety and avoid spoilage during storage (Martelli et al., 2020). One is reformulation, which has been widely used to remove, reduce, increase, add and/or replace different bioactive

components and obtain specific meat-based designs (Cofrades et al., 2017). In recent years, consumers demand meat products that contain preservatives of natural origin to counteract the potential side effects of synthetic additives applied to prolong the storage stability of meat and meat products (Tajkarimi et al., 2010). In this regard, there has been a significant interest in the functional properties of seaweeds/seaweed extracts and their application as bioactive components in the preparation of ingredients and additives that improve the conservation of different types of foods (Ortiz-Viedma et al., 2021). The incorporation of natural additives (i.e. seaweeds) into meat and meat products could be a suitable alternative to retard lipid oxidation but also possibly decrease or prevent adverse colour change (Pindi et al., 2017).

Algae are able to generate natural antioxidants including polyphenols, vitamins (folic acid, ascorbic acid and retinol) and polyunsaturated fatty acids (Vadalà and Palmieri, 2015). The antioxidant activity of natural bioactive compounds from these algae has generated significant interest. In particular, the use of red algae which are rich in bioactive compounds such as polyphenols, flavonoids, tannins, carotenoids and sulphated galactans (Aziz et al., 2021; Ortiz-Viedma et al., 2021), and brown algae who's antioxidant properties are associated with polyphenolic compounds, such as phlorotannins (Aminina et al., 2020). Seaweeds thus are an ingredient with considerable potential for use in the development of functional foods and as a preservation of food, such as processed meat products (Garicano-Vilar et al., 2020). Recent research has demonstrated that red, green or brown seaweeds or seaweed extracts have potential as preservatives in meat and meat products (Gullón et al., 2020). However, despite the growing interest in seaweeds or their extracts as a source of biologically active compounds, their application remains under-exploited.

The analysis of VOC and semi-volatile organic compounds (SVOC) in products such as processed meats is a tool that can be used in product development and quality control as it can provide detailed information on compounds that can positively or negatively impact on sensory perception. In beef, ~880 volatile components have been identified, but only 25 have meaty odours (Miller, 2014). Sulphurous- and carbonyl-containing volatile compounds are thought to be mainly responsible for positive and negative flavour aromatics in meat (Garicano Vilar et al.,

2022). High-capacity sorptive (HiSorb) extraction is a novel technique to extract VOC and SVOC from samples prior to the analysis by thermal desorption gas chromatography–mass spectrometry (TD GC-MS) (Cheng et al., 2021). VOC and SVOC profiling although very beneficial cannot determine the significance of individual compounds in relation to aroma perception, this can only be achieved by GC-O. In GC-O extracted VOC and SVOC eluting from the gas chromatography (GC) column are split between a detector (mass spectrometer or flame ionization) and a sniff port, where trained evaluators can ascribe aroma's and event intensities to individual or co-eluting compounds (Miller, 2014).

Thus, the aims of this study were to apply headspace high capacity sorptive extraction (HiSorb) GC-MS and GC-O to profile volatiles and identify aroma active volatiles, monitor colour and oxidation in raw beef patties reformulated with seaweeds, packed in MAP, and stored under refrigeration conditions for 14 days.

6.3 Material and methods

6.3.1. Meat and seaweed material

Fresh beef (85 % visible lean) was purchased from Ballyburden Meat Processors (Cork, Co. Cork, Ireland) and minced twice through a plate with 4 mm holes (Model P114L, Talsa, Valencia, Spain). The meat was stored at 4 °C until required for analysis and opened before the use by date.

Wild Irish Seaweed Ltd (Quilty, Co. Clare, Ireland) supplied the edible seaweed, Sea Spaghetti– *Himanthalia elongata* (brown species) and Nori– *Porphyra umbilicalis* (red species). The seaweeds were harvested along the west coast of Ireland, air-dried and dehumidified (Caherush Point, Spanish Point, Co. Clare, Ireland). Drying extends the shelf life of seaweed by decreasing the amount of water available for microorganisms (Choe and Oh, 2013). These seaweeds were chosen, because of their composition, antioxidant activity, potential health implications, and abundance on Irish coasts, relatively widespread consumption/acceptability, and suitability for use as ingredients in these processed meat products.

6.3.2. Sample preparation

Dried seaweeds were milled to a particle size of 1 mm by means of a mechanical grinder. Minced beef with 1 % and 3 % milled seaweed (w/w) was formed into 100 g patties using a meat former (Ministeak Burger maker, O.L. Smith Co. Ltd., Italy). The patties were placed in low oxygen permeable (<1 $cm^3/m^2/24$ h at standard temperature and pressure (STP)) polystyrene/ethyl vinyl alcohol/polyethylene trays, using MAP technology. The trays were flushed with 80 % O_2: 20 % CO_2 using a vacuum-sealing unit (VS 100, Gustav Müller and Co. KG, Bad Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH and Co. KG, Witten, Germany). Trays were covered and heat-sealed using a low oxygen permeable (3 $cm^3/m^2/24$ h at STP) laminated barrier film with a polyolefin heat-sealable layer and subsequently stored for 14 days at 4 °C. The gas atmosphere (O_2 % and CO_2 %) in the packs was checked at the beginning and at the end of the packaging trial using a gas analyser (CheckMate 9900, PBI-DanSensor, Denmark) where the instrument needle was inserted through the lidding material.

6.3.3. Proximate analysis

A CEM Smart and a SMART Trac system (CEM GmbH, Kamp-Lintfort, Germany) were used to measure the moisture and fat content, respectively, of the control and reformulated beef patties (Leffler et al., 2008).

6.3.4. Colour measurement

The surface colour of the control and reformulated beef patties was measured using a Konica Minolta CR-400 Chroma Meter (Minolta Camera Co., Osaka, Japan). Six readings were taken per storage time and averaged for statistical analysis. The Chroma-Meter consisted of a measuring head (CR-400), with an 8 mm diameter measuring area, a 2° standard observer, and a data processor (DP-400). The Chroma Meter was calibrated on the CIE LAB colour space system using a white tile (L* =

94.31, a* = -0.45, b* = 3.90). The 'L*' value represents lightness (L*=0 yields black and L*=100 indicates diffuse white) and 'a*' and 'b*' values represent redness and yellowness, respectively (a*, negative values indicate green while positive values indicate magenta and b*, negative values indicate blue and positive values indicate yellow). a* values are the most important colour parameter for fresh meat (Olivera et al., 2013). Delta E 2000 formula was used to measure the visible difference between two colours, in this case between samples from day 0, 7 and 14 of storage. ColorTools Excel add-in was used to apply the formula. A smaller Delta E value (ΔE*) represents a closer colour match.

6.3.5. Microbiological analysis

The microbial analysis of the patties was undertaken by ALS Life Sciences Ltd. (Clonmel, Co. Tipperary, Ireland). The control and reformulated beef patties were tested for *enterobacteriaceae* (ISO 21528-2: 2017), aerobic colony count (ISO 4833-1: 2013) and ß-glucuronidase + *E. coli* (ISO 16649-2: 2001). Microbiological analysis of the patties was carried out on days 3, 7 and 14 of storage. The results were expressed as logarithms of colony-forming units per gram of sample (log CFU/g).

6.3.6. Total phenolic content and *in vitro* antioxidant activity of seaweed

The TPC, the free radical scavenging activity (RSA) and the total antioxidant capacity (TAC) of the seaweed extracts were determined as described in Mohammed et al. (Mohammed et al., 2021). Polyphenol rich extracts were prepared. Brown and red seaweeds (0.5 g) were placed in tubes with 20 ml of methanol/water (50:50). The pH was adjusted to 2.0 using HCl, and tubes were shaken in a water bath (Julabo SW 23, Julabo GmbH, Seelbach, Germany) at 200 rpm for 1 h at 20 °C. Tubes were centrifuged at 4000 rpm for 10 min, and the supernatants were recovered. Acetone/water (70:30) (20 ml) was added to the supernatant residues, and the shaking and centrifugation steps were repeated. Supernatants from both extractions were combined. The TPC were measured using the Folin–Ciocalteu method

(Singleton and Rossi, 1965); the RSA was determined according to the DPPH assay (Yen and Wu, 1999) and the TAC was measured using the FRAP method (Benzie and Strain, 1999). TPC results were expressed as milligrams of gallic acid equivalents (GAE)/g of seaweed, and DPPH results and FRAP results were expressed as milligrams of Trolox equivalent/g seaweed.

6.3.7. Lipid oxidation measurement

Lipid oxidation was measured in the patties using the 2-thiobarbituric acid (TBA) assay as described by Siu and Draper (1978) (Siu and Draper, 1978). Chopped beef samples (5 g) were homogenised for 1.5 min in 25 ml distilled water using an Ultra Turrax tissue homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen, Germany). Trichloroacetic acid (TCA) 10 % was added (25 ml) and the mixture was shaken vigorously and filtered through Whatman No. 1 filter paper. In screw-capped test tubes, 4 ml of clear filtrate was added to 1 ml of 0.06 M TBA. The tubes were placed in a water bath and held at 80 °C for 90 min. The absorbance of the filtrate was measured spectrophotometrically (Cary 300 Bio, UV-vis spectrophotometer, Varian Instruments, CA, USA) at 532 nm against a blank containing all reagents (2 ml distilled water, 2 ml 10 % TCA and 1 ml of 0.06 M TBA reagent). The malondialdehyde (MDA) content of the samples was calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}. Results were expressed as 2-thiobarbituric acid-reactive substances (TBARS) in mg MDA/kg beef.

6.3.8. Extraction and volatile compounds analysis

6.3.8.1. Standard solution

Internal standard stock solution was prepared at 0.1 % (w/v), containing 123 µl 4-methyl-2-pentanol and 122 µl 2-methyl-3-heptanone in 100 ml methanol, and was stored at -18 °C until required. The internal standard mix was prepared with 100 µl

of internal standard stock solution in 10 ml distilled water (10 ppm). One hundred µl of internal standard mix was added to each sample.

6.3.8.2. Probe-based high-capacity sorptive extraction (HiSorb) parameters

The meat samples were taken out of the fridge (4 °C) and allowed to adjust to room temperature. The meat was then cut into smaller pieces manually and weighed (1 g). The cut meat was placed into 20 ml clear glass headspace vials with crimp seals/top with prefitted polytetrafluoroethylene (PTFE) septa (Markes International Ltd., Bridgend, UK). Internal standard mix (100 µl) was added to each sample. An easy grip manual crimper for aluminium caps was used to seal the vials (Restek™ Corporation, Bellefonte, PA, USA). Samples were prepared in triplicate. Fifteen replicates were analysed, three contained beef sample + 100 µl of internal standard mix (n = 3); six contained beef sample with 1 % (n = 3) and 3 % (n = 3) Sea Spaghetti– *H. elongata* + 100 µl of internal standard mix, and six contained beef sample with 1 % (n = 3) and 3 % (n = 3) Nori– *P. umbilicalis* + 100 µl of internal standard mix.

The extraction of 1 g of beef sample with/without seaweed by HiSorb was performed as previously optimized in Garicano Vilar et al. (see chapter 5) for 180 min at 40 °C. A PDMS HiSorb probe (Markes International Ltd., Bridgend, UK), consisting of a short section of PDMS located at the end of a stainless-steel probe, was inserted into the headspace of the 20 ml vial through the HiSorb cap/septum (C-HSCSC-20C-50). The vials were then placed in a HiSorb Agitator (U-HSAG-20) (Markes International Ltd., Bridgend, UK) for agitation and gentle heating to ensure that analytes from the sample are effectively absorbed into the sorbent volume. Once the extraction was complete, the HiSorb probes were removed from the sample vial, rinsed with distilled water to remove any residue from the surface, dried with a lint-free tissue and inserted into a TD tube for desorption. All samples were extracted in triplicate.

6.3.8.3. Volatile compounds analysis by gas chromatography-mass spectrometry

Following extraction, a Unity 2 Thermal Desorption Unit (Markes International Ltd., UK), connected to an autosampler, was used to concentrate the volatiles and remove any excess moisture prior to direct transfer to the GC-MS. The Unity 2 was operated with nitrogen as the purge gas at 50 psi at a flow rate of 1.2 ml/min. Tubes were dry purged for 2 min using a 1:20 split. A two-stage desorption was employed: the first stage desorption was performed at 150 °C and held for 5 min, the second stage desorption was performed at 300 °C and held for 5 min. A 1:10 split was used for final tube desorption. The trap used was a materials emissions trap and this was held at 30 °C during tube desorption with a gas flow of 50 ml/min. Prior to trap desorption, a 2 min pre-trap fire purge was performed with a 1:50 split. Trap desorption was performed at 30-300 °C at a rate of 24 °C/min and held for 5 min with 1:10 split.

The GC-MS system was an Agilent 7890A GC with an Agilent 5977B mass spectrometry detector (MSD) (Agilent Technologies Ltd., Cork, Ireland). The column used was a capillary DB-624 UI (60 m x 0.3 mm x 1.8 µm) (Agilent Technologies Ltd., Ireland) with helium as the carrier gas. The column temperature started at 40 °C held for 5 min, increased to 230 °C at 5 °C/min and held at 230 °C for 35 min. The total run time was 78 min. Injector temperature was set at 250 °C. Desorbed volatile compounds were injected in splitless mode with a consistent pressure of 23 psi. The mass spectra of VOC were generated by a mass spectrometer (MS) quadrupole detector with an ionisation voltage of 70 eV, 3.32 scans/s and a scanning mass range of 35-350 amu. The ion source temperature was 220 °C and the interface temperature was set at 280 °C. Autotunes were carried out weekly.

Volatile compounds were identified by comparing their mass spectra to the National Institute of Standards and Technology (NIST) 2014 mass spectral library and an in-house library created using authentic external compounds with target and qualifier ions for each compound, using MassHunter Qualitative Analysis Software (Agilent Technologies, Palo Alto, CA, USA). Spectral deconvolution was also

performed to allow the identification of untargeted and co-eluting compounds using Automatic Mass Spectral Deconvolution and Identification System (AMDIS) (http://chemdata.nist.gov/mass-spc/amdis/). Linear retention indices (LRI) were determined using the method by Van Den Dool and Kratz (van Den Dool and Kratz, 1963) and matched against peer reviewed publications (Corral et al., 2015; Gallego et al., 2012; Gianelli et al., 2011). Positive identification was only attained once the mass spectral match reached >80 %, using external standards where possible and when LRI were within 10 % of referenced values. The results were expressed in abundance values. Quantification of each compound was carried out using calibration curves and the relevant standard values.

6.3.9. Gas chromatography-olfactometry

GC-O analysis was carried out on twenty-five samples (n = 25; n=5 controls, n=5 Sea Spaghetti 1 %, n=5 Sea Spaghetti 3 %, n=5 Nori 1 %, n=5 Nori 3 %) after extraction by HiSorb. GC-O was conducted on an Agilent 7890A GC coupled with an Agilent 5975C MSD (Agilent Technologies Ltd, Cork, Ireland). The GC was equipped with a flame ionization detector (FID) and a Gerstel Olfactory Detection Port ODP3 with heated mixing chamber (Anatune Ltd, Cambridge, UK). A humidifying device was used to reduce nasal mucosa dehydration. Analyses were performed on a DB-624 column (20 m x 1.80 mm i.d. x 1 µm phase thickness). Helium was used as a carrier gas at a flow rate of 1.2 ml/min and nitrogen as auxiliary gas. The GC oven temperature was programmed to increase from 60 to 180 °C at a rate of 6 °C/min, with an initial hold time of 2 min, and from 180 to 220 °C at a rate of 15 °C/min, with a final hold time of 5 min. The total run time was ~30 min. Column effluent was split equally between the FID, the olfactory port and the MSD. The transfer line into the MSD was kept at 260 °C. The FID temperature was set at 300 °C, with an air flow of 400 ml/min, a H_2 fuel flow of 30 ml/min and a N_2 makeup flow of 25 ml/min. The sniffing port and its exit were maintained at 100 °C and 40 °C, respectively.

A panel of five trained sensorial assessors carried out the sniffing of the volatile compounds of the control and reformulated beef patties extracted by HiSorb. The

panellist determined descriptors of beef and seaweed aromas and developed standardized references that could be used to uniformly and consistently identify samples attributes prior to the analysis. None of the assessors were anosmic, as tested by Sniffin' Sticks test (Burghardt Messtechnik, Wedel, Germany) prior to the GC-O analysis (Rumeau et al., 2016). Sniffing time was approximately 30 min and each assessor carried out one session per day. The panellists were asked to rate: 1) the intensity of the eluted aroma using a four-point category scale (1= weak, hardly recognizable odour; 2= clear but not intense odour, 3= intense odour, 4= very intense odour), recorded by a Gerstel OID Interface/ODP-Recorder (Anatune Ltd, Cambridge, UK) and 2) the odour perceived, by voice recording. The odorants taken as significant were the ones in which at least two assessors were able to detect.

Tentative identifications were based on comparison of the mass spectra of unknown compounds against those of the NIST, and by comparing the retention index value to values available in the literature and in an in-house library. The odorants were also identified by comparison of their odours with Flavornet database [http://www.flavornet.org], The Good Scents Company database [http://www.thegoodscentscompany.com] and other specified publications. Retention indices for all detected volatile compounds were calculated using an n-alkane series (Merck, Arklow, Ireland) for both GC-MS and GC-O analysis.

6.3.10. Data analysis

Colour changes, changes in VOC abundances and changes in aroma intensities were evaluated by Bonferroni multiple comparison analysis (one-way ANOVA), using statistical package for the social sciences (SPSS) software version 24 (IBM Corp., Armonk, NY) to determine if there were statistically significant differences ($P < 0.05$) across storage time. R software (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) for statistical computing and graphics was used to analyse the volatile component profile of the samples using the package metaMS (Wehrens et al., 2014). A hierarchical clustering heatmap with the abundance values

of volatile compounds derived from each sample at different storage time points was generated using the R package pheatmap (Kolde, 2019); alongside a principal component analysis (PCA) scores plot depicting the distribution of volatile compounds in regards of days of storage using the packages FactoMineR (Lê et al., 2008) and Factoextra (Kassambara and Mundt, 2020).

6.4 Results and discussion

6.4.1. Gas and proximate analysis

Control and reformulated beef patties MAP trays contained 76.8 % O_2 and 21.8 % CO_2 at day 0, and 76.1 % O_2 and 21.8 % CO_2 at day 14 (end of storage). The fat content of fresh beef patties (day 0) was 13.6 ± 0.31 %, whereas moisture content was 66.6 ± 0.61 % (Table S-6.1).

6.4.2. Colour measurement

During refrigeration, the colour of the raw meat changes as myoglobin forms oxymyoglobin, then metmyoglobin due to continued exposure to oxygen and light that eventually turns meat into a undesirable brownish-red colour (U.S. Department of Agriculture, 2013). Seaweed also experiences colour changes during drying and storage. Harrysson et al. (Harrysson et al., 2021) observed an increase in L* (lightness) and a* values (loss of green colour), and a decrease in b* value (loss of yellow colour), particularly for seaweeds stored in light conditions. Similarly, Oh et al. (Oh et al., 2014) found a significant decrease in both chlorophyll (green colour) and carotenoids (yellow colour) in seaweed after 15 days of storage in both darkness and light, with greater losses in the latter.

L* values (lightness) were found to be significantly higher (P <0.001) as time passed in the Control, 3 % Sea Spaghetti and Nori samples (Table 6.1). a* values (redness) decreased significantly (P <0.001) with increasing storage time in all samples; the magnitude of difference between day 0 and day 7 was higher than that

observed between day 7 and day 14 (Table 6.1). The b* values (increment of blue, loss of yellow) were only significantly higher (P <0.001) in Nori samples and significantly lower (P = 0.046) in 3 % Sea Spaghetti samples compared to the control. Total colour change was calculated using Delta E (ΔE*) to study the differences in colour of the beef patties across storage time. The higher ΔE*, the further the colour was from the baseline hue. The baseline reference was the colour of the patties at day 0 of storage. The value of ΔE* increased as storage time increased, except in 3 % Sea Spaghetti samples (Table 6.1) possibly due to a slight bleaching effect. If ΔE* is less than 1 between two colours it is barely perceivable by the average human observer (Dattner and Bohn, 2015), but when ΔE* is higher than 3 colour differences are obvious (Francis and Clydesdale, 1977). Hence, samples at day 7 and at day 14 were perceived as having a significant colour deviation. Similar observations were made in Olivera et al. (Olivera et al., 2013) and Ijaz et al. (Ijaz et al., 2020), where colour changes were perceived from day 1 to day 3 in dark for dry beef stored at 4 °C for 7 days.

Table 6.1. Colour changes of control and reformulated beef patties under MAP throughout storage time.

	Day 0	**Day 7**	**Day 14**		**Day 0**	**Day 7**	**Day 14**	
		M ± SD		*P*-value		M ± SD		*P*-value
Control								
L*	46.04 ± 1.18^{a}	53.66 ± 3.85^{b}	57.14 ± 2.48^{b}	<0.001	-	-	-	-
a*	23.62 ± 0.98^{a}	5.83 ± 0.73^{b}	4.02 ± 0.38^{c}	<0.001	-	-	-	-
b*	13.98 ± 1.02^{a}	13.79 ± 0.75^{a}	14.63 ± 0.65^{a}	0.214	-	-	-	-
ΔE*	N/A	16.01	19.71	N/A	-	-	-	-
Sea Spaghetti		**1 %**				**3 %**		
L*	47.22 ± 4.90^{a}	51.74 ± 2.33^{a}	51.30 ± 1.79^{a}	0.06	40.92 ± 2.15^{a}	49.44 ± 4.83^{b}	41.92 ± 5.80^{a}	0.01
a*	21.39 ± 1.60^{a}	4.39 ± 0.63^{b}	3.20 ± 0.38^{b}	<0.001	18.89 ± 1.74^{a}	1.89 ± 1.54^{b}	4.25 ± 1.06^{c}	<0.001
b*	13.77 ± 0.62^{a}	13.63 ± 0.57^{a}	14.18 ± 0.66^{a}	0.310	12.25 ± 0.61^{a}	11.80 ± 1.03^{a}	10.23 ± 1.97^{a}	0.046
ΔE*	N/A	15.22	16.59	N/A	N/A	18.10	12.80	N/A
Nori		**1 %**				**3 %**		
L*	38.47 ± 2.87^{a}	48.26 ± 3.97^{b}	55.16 ± 2.22^{c}	<0.001	33.40 ± 6.25^{a}	42.38 ± 6.78^{b}	49.91 ± 2.83^{b}	<0.001
a*	15.26 ± 2.75^{a}	4.45 ± 0.69^{b}	1.15 ± 0.61^{c}	<0.001	6.05 ± 2.26^{a}	4.84 ± 0.46^{a}	1.47 ± 0.44^{b}	<0.001
b*	10.89 ± 1.71^{a}	11.49 ± 0.24^{a}	14.05 ± 0.66^{b}	<0.001	6.53 ± 1.76^{a}	7.94 ± 2.16^{a}	12.09 ± 0.85^{b}	<0.001
ΔE*	N/A	14.00	22.51	N/A	N/A	7.94	16.82	N/A

M, mean; SD, standard deviation; N/A, not applicable; *P* <0.05 considered statistically significant; $^{a–c}$, means in the same row bearing different superscript letter denote statistically significant differences (*P* <0.05).

6.4.3. Microbiological analysis

There has been growing concern about meat products carrying pathogenic microorganisms (Kim et al., 2018). However, the complete elimination of pathogens from raw meat is difficult or impossible, despite enhanced efforts in meat and processed meat hygiene (Kim et al., 2018). For raw meat products, safety and quality can be estimated by the use of indicator microorganisms, including *Enterobacteriaceae* (Mladenović et al., 2021), total aerobic colony count (ACC) and *Escherichia coli* count (ECC) (Kim and Yim, 2016). *Enterobacteriaceae* are considered the indicator bacteria for microbiological quality of food and hygiene status of a production process. Higher levels of *Enterobacteriaceae* (>10^4 CFU/g) suggests an overall poor general hygiene status of a food product (Health Protection Agency, 2009) and could pose a microbiological risk for consumers (Mladenović et al., 2021). ACC provides an estimate of overall bacterial populations. A higher ACC usually relates to poorer quality and a reduced shelf life. ECC provides an estimate of poor sanitation during processing. High ECC generally correlates with higher levels of foodborne pathogens originating from faeces (Kim and Yim, 2016). Low levels of *E. coli* may occasionally be found in raw meat, however, it should not be detected in ready-to-eat foods (Health Protection Agency, 2009).

Different studies have attributed an antimicrobial capacity to seaweed extracts due to the presence of bioactive compounds such as phlorotannins (Eom et al., 2012), flavonoids, steroids, and sulfated polysaccharides (52,53). Many of these compounds have been proven to be able to inhibit bacterial growth (Hintz et al., 2015), particularly *E.coli* (Plaza et al., 2010) and other bacteria (Silva et al., 2020) in *H. elongata* and Gram-positive and Gram-negative bacteria in *Porphyra* sp. due to compounds such as porphyran (sulphated polysaccharide) (Bhatia et al., 2015). Cox and Abu-Ghannam (Cox and Abu-Ghannam, 2013) observed lower microbiological counts in cooked beef patties with *H. elongata* powder stored for 30 days in refrigeration.

According to the Health Protection Agency guidelines for assessing microbiological safety of ready-to-eat foods (Health Protection Agency, 2009), our

mean ACC levels were within borderline quality (10^6 - $<10^8$ CFU/g) through storage (Table 6.2). *Enterobacteriaceae* count only reached unsatisfactory levels ($>10^4$ CFU/g) at day 14 (Table 6.2). ß-glucuronidase + *E. coli* levels remained satisfactory (<20 CFU/g) throughout storage (Table 6.2).

Table 6.2. Microbial counts (CFU/g) of control and reformulated beef patties (1 % and 3 % seaweed addition) across storage time.

	Day 3		**Day 7**		**Day 14**	
	CFU/g		CFU/g		CFU/g	
Control						
Enterobacteriaceae	7100		4100		>15000	
Aerobic colony count	2700000		>30000000		>30000000	
ß-Glucuronidase + *E. coli*	<10		<10		<10	
Sea Spaghetti	**1 %**	**3 %**	**1 %**	**3 %**	**1 %**	**3 %**
Enterobacteriaceae	4200	880	3100	2800	>15000	>15000
Aerobic colony count	3100000	2200000	>30000000	>30000000	>30000000	>30000000
ß-Glucuronidase + *E. coli*	<10	<10	<10	<10	10	10
Nori	**1 %**	**3%**	**1 %**	**3 %**	**1 %**	**3 %**
Enterobacteriaceae	1500	2000	4700	2700	>15000	>15000
Aerobic colony count	15000000	25000000	>30000000	>30000000	>30000000	>30000000
ß-Glucuronidase + *E. coli*	<10	<10	<10	<10	10	<10

CFU, colony-forming unit.

6.4.4. Total phenolic content and *in vitro* antioxidant activity

The major groups of antioxidant compounds in macroalgae that make them promising species for food use include carotenoids, phenolic compounds, phycobilin pigments, polyphenols, sulphated polysaccharides and vitamins (ascorbate and vitamin A) (Cornish and Garbary, 2010).

Sea Spaghetti had higher (P <0.05) TPC than Nori (Table 6.3). Comparable TPC levels in Sea Spaghetti (10.7 ± 0.5 mg GAE/g extract), slightly higher in Sea Spaghetti (18 ± 2 mg GAE/g seaweed), lower in Nori (2.9 ± 0.07 mg GAE/g seaweed) have been previously reported by Gomes et al. (Gomes et al., 2022) and Fernández-Segovia et al. (Fernández-Segovia et al., 2018), respectively.

DPPH and FRAP activities between Sea Spaghetti and Nori were not statistically significant (P >0.05) (Table 6.3) and similar DPPH values were reported by Gomes et al. (Gomes et al., 2022) for Sea Spaghetti. However, Fernandez-Segovia et al. (Fernández-Segovia et al., 2018) observed statistically higher (P <0.001) FRAP activity in Sea Spaghetti (41.1 ± 0.8 µmol trolox/g) than in Nori (2.1 ± 0.2 µmol trolox/g). Our results agree with a study carried out on meat products incorporating seaweeds, which had demonstrated a greater antioxidant capacity for products with Sea Spaghetti (3.69 µmol trolox/g meat product), than with Nori (1.18 µmol trolox/g meat product), but no significant antioxidant differences were found between them (López-López et al., 2009).

Table 6.3. Total phenolic content and in vitro antioxidant activity of Sea Spaghetti– *Himanthalia elongata* and Nori– *Porphyra umbilicalis*.

	TPC (mg GAE / g)*	**Antioxidant capacity (mg trolox / g)**	
		DPPH	**FRAP**
	M ± SD	M ± SD	M ± SD
Sea Spaghetti	12.74 ± 0.43	3.65 ± 0.17	4.66 ± 0.16
Nori	8.57 ± 0.10	3.45 ± 0.15	4.46 ± 0.15

TPC, total phenolic content; GAE, gallic acid equivalent; DPPH, 2,2-diphenyl-1-picryl-hydrazyl-hydrate; FRAP, ferric reducing antioxidant power. M, mean of three different batches each analysed in triplicate; SD, standard deviation. * P <0.05. (Adapted from Mohammed et al. (Mohammed et al., 2021)).

6.4.5. Lipid oxidation measurement

Lipids are one of the most chemically unstable food components (Min and Ahn, 2005). Lipid oxidation is a major cause of meat quality deterioration (Shimizu and Iwamoto, 2022), that affects the nutritional value, colour, texture, taste and aroma of meat, leading to rancidity (Amaral et al., 2018). The oxidation reaction not only impacts fatty acids causing rancidity, but can also co-oxidize pigments and vitamins; reducing both the nutritional and sensorial quality of foods (Harrysson et al., 2021). The degree of lipid oxidation in meat is often quantified using TBARS (Zhang et al., 2019).

Suppressing lipid oxidation is important for optimising shelf life (Shimizu and Iwamoto, 2022). Radical scavengers and chelating agents are known as antioxidants, and suppress the progression of lipid oxidation. Many studies have already shown the antioxidant and antimicrobial activity of polyphenols extracted from *H. elongata* by in vitro assays (Rajauria et al., 2016). Flavonoids are thought to exert beneficial effects through their antioxidant and chelating properties, and are the major contributor to the antioxidant capacity (Rajauria et al., 2013). They act either by blocking the generation of hypervalent metal forms, by scavenging free radicals, or by breaking lipid peroxidation chain reactions (Zaragozá et al., 2008). *Porphyra* species also contain antioxidants such as polyphenols, tocopherols, and porphyran, which contribute to lipid oxidative stability (Oh et al., 2014).

Figure 6.1 shows the TBARS values of raw beef and reformulated beef patties with seaweed over storage. Overall the TBARS level was 1.066 ± 0.17 mg MDA/kg at day 0. The mean TBARS levels continued to increase to 6.193 mg MDA/kg on day 7 and 6.970 mg MDA/kg on day 14 of storage. TBARS content increased throughout storage, as previously reported (Faustman et al., 2010; Quevedo et al., 2013; Wilkinson et al., 2001). Min et al. (Min et al., 2008) suggested that the TBARS values of raw beef increased significantly during storage because of a high heme iron content, high lipoxygenase-like activity, and a low DPPH radical scavenging activity.

On day 14 of storage, trends indicated significantly lower levels of lipid oxidation in beef patties containing 3 % Sea Spaghetti (P <0.001) or 3 % Nori (P =

0.003) compared to the control (Fig. 6.1). It can also be seen that the antioxidant capacity on day 14 among the beef patties formulated with Sea Spaghetti was significantly higher than those formulated with Nori (P = 0.004 1 %; P = 0.007 3 %) (Fig. 6.1). The greater antioxidant capacity of Sea Spaghetti (Table 6.3) is likely due to the higher TPC content. However, as the seaweed was added to the beef in dried form this may have adversely affected the antioxidant capacity (Cabral et al., 2021); for example extraction using water or ethanol/water as the solvent can concentrate the antioxidant compounds (Rajauria et al., 2016). An example of this is Agregan et al. (Agregán et al., 2019), who observed that TBARS levels of pork patties containing 1000 mg *Fucus vesiculosus* extract/kg were lower than those obtained for the control sample after 18 days of storage. Nevertheless, Cox and Abu-Ghannam (Cox and Abu-Ghannam, 2013) also observed inhibition of lipid oxidation (significantly lower TBARS levels (38-45 %)) when *H. elongata* powder was added to cooked beef patties in comparison to a control formulation. The authors justified this increased lipid stability due to the phenolic compounds with high DDPH activity present in the Sea Spaghetti seaweed.

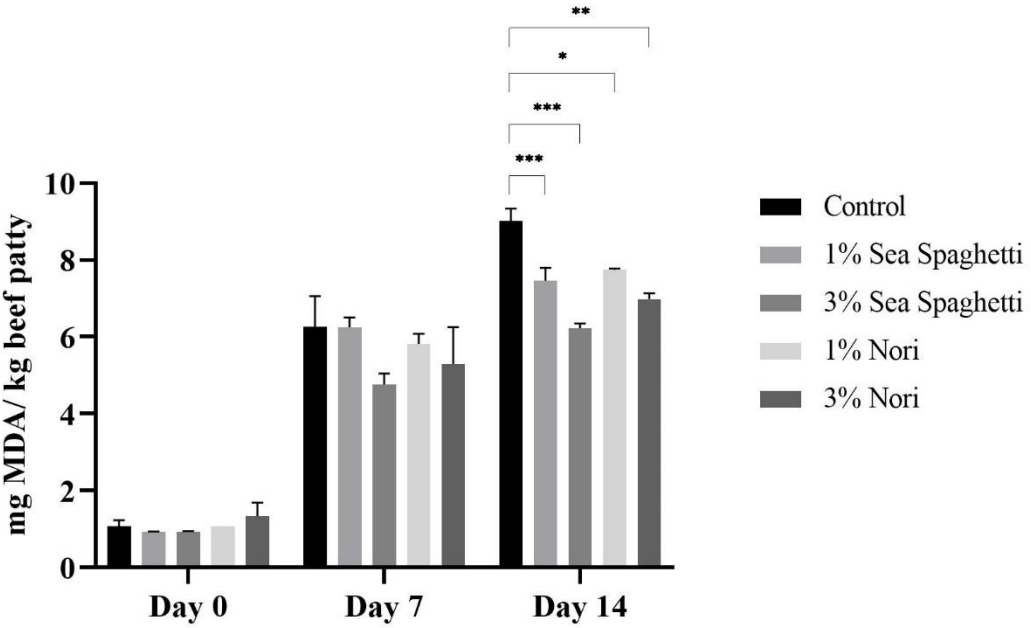


Figure 6.1. Lipid oxidation of control and reformulated beef patties during storage measured by TBARS (mg malondialdehyde/kg beef). * P <0.05; ** P <0.01; *** P <0.001.

A true antioxidant effect would have been considered where oxidation was reduced to a level below what is considered acceptable for organoleptic detection (i.e. 1-2 mg MDA/kg sample). Some previous studies have suggested TBARS limits in beef for consumer acceptance would be 1.0 mg MDA/kg (McKenna et al., 2005) or 2.0 mg MDA/kg (Campo et al., 2006). Based on this, the beef patties could not be considered suitable for human consumption at day 7 or 14 of storage (>6 mg MDA/kg) and no true antioxidant effect could be ascribed to the seaweed addition (Fig. 6.1). It is necessary to take into account that the strength of antioxidant ability depends on the concentration of the antioxidant compounds (Alkhalaf, 2021). Thus, seaweed addition if optimised may still have an antioxidant role, but the species, format and dosage would also have to be assessed, as they affect other parameters in the final product that may impact on consumer acceptance.

6.4.6. Volatile compounds analysis

Several studies have formulated seaweed-enriched meat products (Choi et al., 2016; Cox and Abu-Ghannam, 2013; Jiménez-Colmenero et al., 2010; López-López et al., 2010; López López et al., 2009), however, to the best of our knowledge, none have analysed their volatile component profile. In this study 84 VOC were identified in total, including 23 aldehydes, 20 alcohols, 13 ketones, 11 acids, 7 alkanes, 4 esters/ethers, 3 furans, 2 benzenes and phenol. Seaweed introduced new VOC that were not present in the control, such as acids (dodecanoic acid and tetradecanoic acid), alcohols (2-butanol and 2-methyl-1-butanol), aldehydes ((E)/-2-hexenal and tridecanal), ketones (cyclohexanone, 2-octanone, acetophenone and (E,E)-3,5-octadien-2-one), a benzene (toluene), and a furan (2-n-butyl furan). The impact of storage time on the abundance of the different VOC is highlighted in the heatmap (Fig. 6.2). Acids, alcohols, aldehydes, furans and ketones increased, both in control

samples and reformulated samples; while alkanes, benzenes and esters decreased in the reformulated samples in comparison to the control, with the exception of esters which increased in the control samples. All compound classes increased with storage time in reformulated samples, with the exception of alkanes and benzenes in the Nori samples, esters in 1 % Sea Spaghetti samples (which decreased after day 7 of storage) and alkanes in 3 % Sea Spaghetti samples.

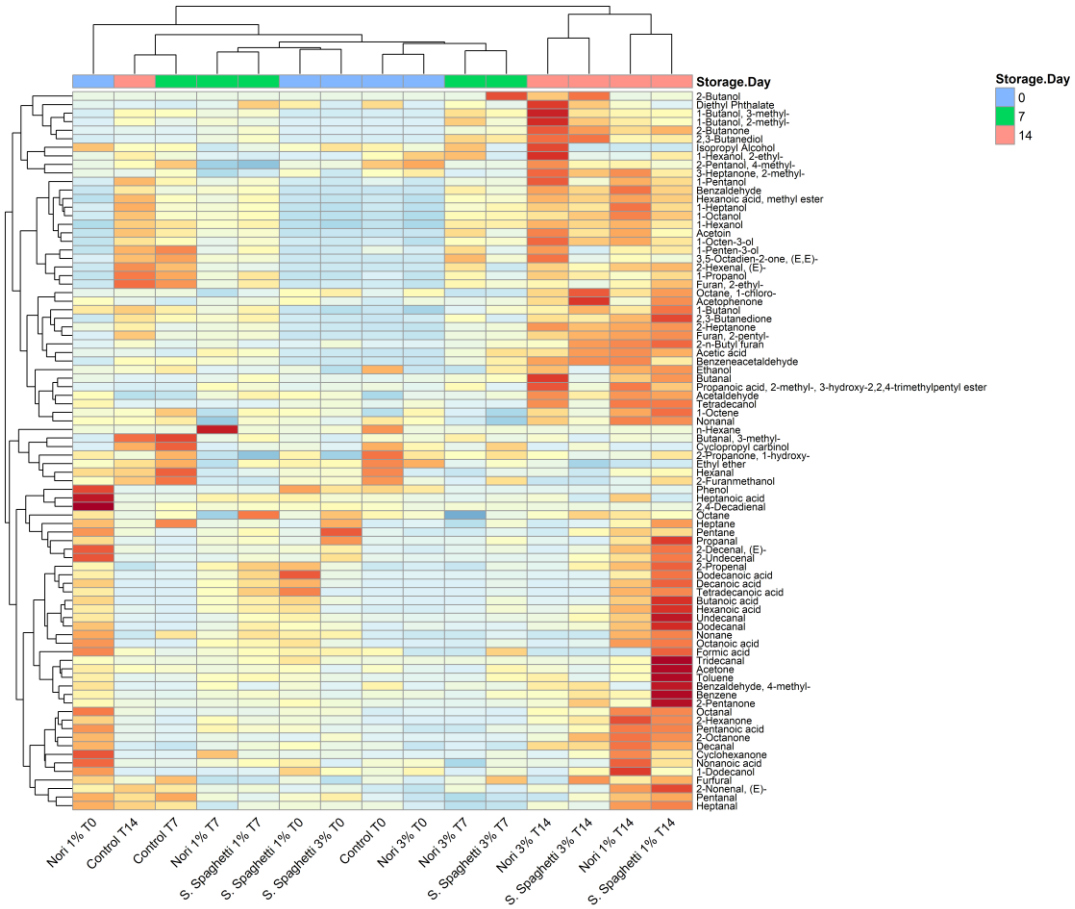


Figure 6.2. Hierarchical clustering heatmap of volatile compounds derived from beef patties and reformulated patties stored in MAP for 14 days. The degraded scale of colour, from red (higher abundance) to blue (lower abundance), reflects the abundance of the identified volatile compounds.

Individual PCA's were generated to visualize the volatile differences between samples regarding the amount of seaweed added (Fig. 6.3A) and the storage time (Fig. 6.3B).

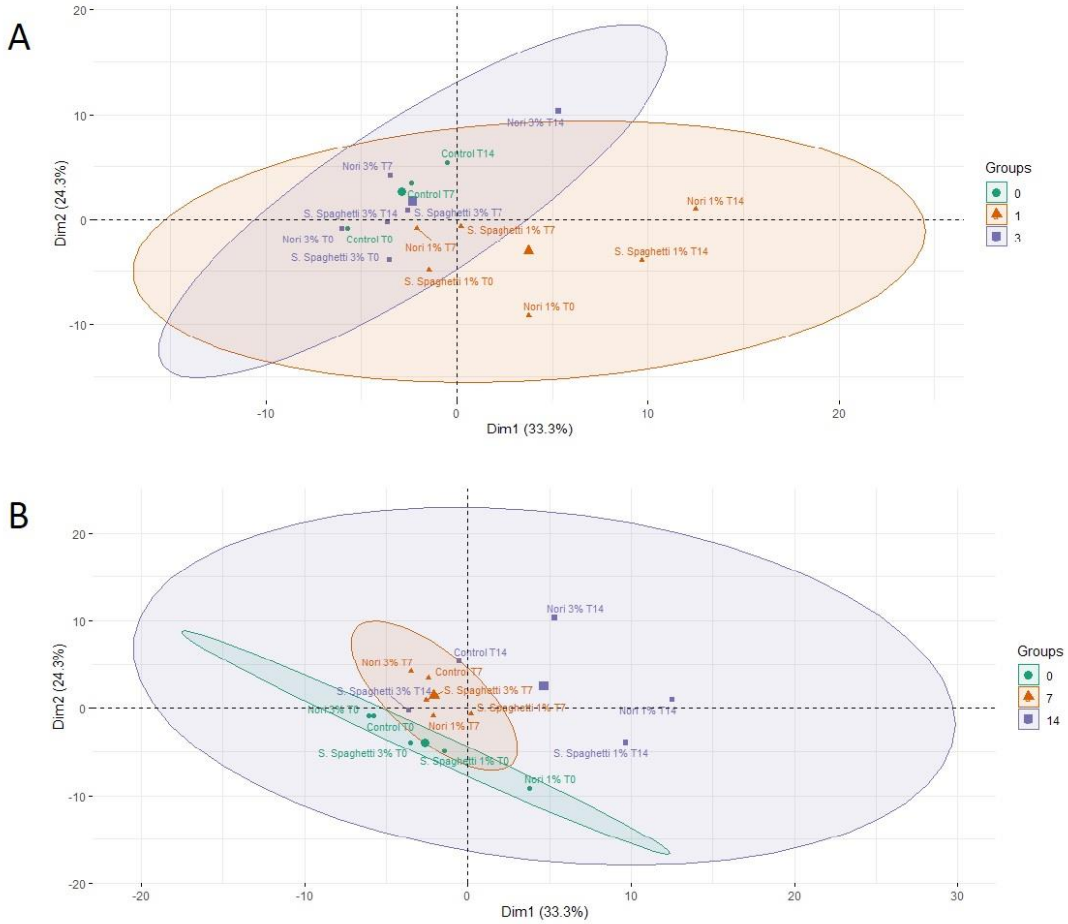


Figure 6.3A and 6.3B. Principal Component Analysis (PCA) plot depicting the grouping of samples with regards to the amount of seaweed addition (A; 0 % green ●, 1 % orange▲, 3 % purple ■) and the storage time (B; 0 days green ●, 7 days orange▲, 14 days purple ■), on the volatile profile for the first two principal components (PC1 and PC2).

The volatile compounds making the greatest contribution to differences between the control and the reformulated patties over storage were the acids (butanoic, pentanoic, hexanoic, octanoic and decanoic acid), selected aldehydes (octanal, undecanal, 2-undecenal, (E)-2-decenal, dodecanal), alcohols (1-octen-3-ol and 1-hexanol), ketones (acetoin, 2-hexanone and 2-heptanone) and 2-pentylfuran, benzaldehyde and hexanoic acid methyl ester (methyl hexanoate) (Fig. 6.4). It is likely

that many of these compounds are directly related to the inclusion of the seaweeds, as the volatile profile of seaweeds can be quite diverse (Garicano Vilar et al., 2020).

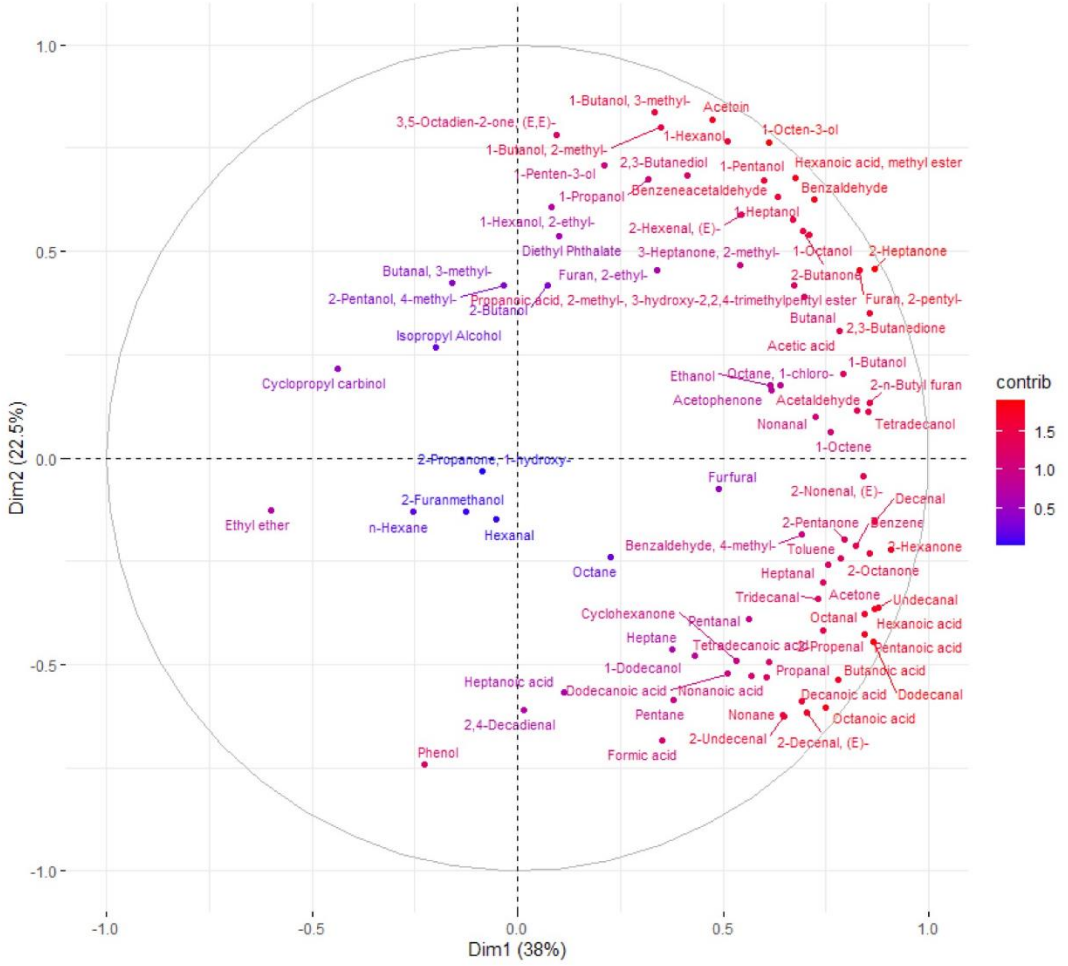


Figure 6.4. Principal Component Analysis (PCA) plot for the volatile compounds making the greatest contribution to the differences between the control and the reformulated patties. The contribution of the 80 most discriminating volatile compounds is reflected in a degraded scale of colour from red (higher contribution) to blue (lower contribution).

Another PCA was obtained to visualize the relationship between the VOC in the patties at different storage times (Fig. 6.5). The first two principal components of the PCA explained 57.6 % of the variance (Fig. 6.5). A clear discrimination exists between the samples based on their storage time (Fig. 6.3B and 6.4). Time point 0 days differed

from the other two time points (T7 and T14) for all samples on the negative side PC1. T0 samples were correlated with ethyl ethanol, phenol, hexanal, 2,4-decadienal, n-hexane, formic acid and heptanoic acid (Fig. 6.5). T7 were quite similar, remaining on the negative side of PC2 and the negative side of PC1, and associated mainly with alcohols such as isopropyl alcohol, cyclopropyl carbinol, 4-methyl-2-pentanol and 2-furanmethanol (Fig. 6.5). T14 samples were mainly on the positive side of PC2, discriminating 1 % seaweed samples from 3 % seaweed samples. At T14 the 1 % seaweed samples were associated with 2-n-butylfuran, tetradecanol, acetaldehyde, nonanal and (E)-2-Nonenal (Nori); and 2-hexanone, 2-octanone, undecanal, dodecanal and hexanoic acid (Sea Spaghetti) (Fig. 6.5). At T14 the 3 % seaweed samples were associated with 3-methyl-1-butanol, 1-hexanol, acetoin and 1-octen-3-ol (Nori); and 2-ethylfuran, 2-butanol, 2-methylpropanoic acid and 2-methyl-3-heptanone (Sea Spaghetti) (Fig. 6.5). It is evident that the addition of the seaweed altered the volatile profile which subsequently changed with storage time.

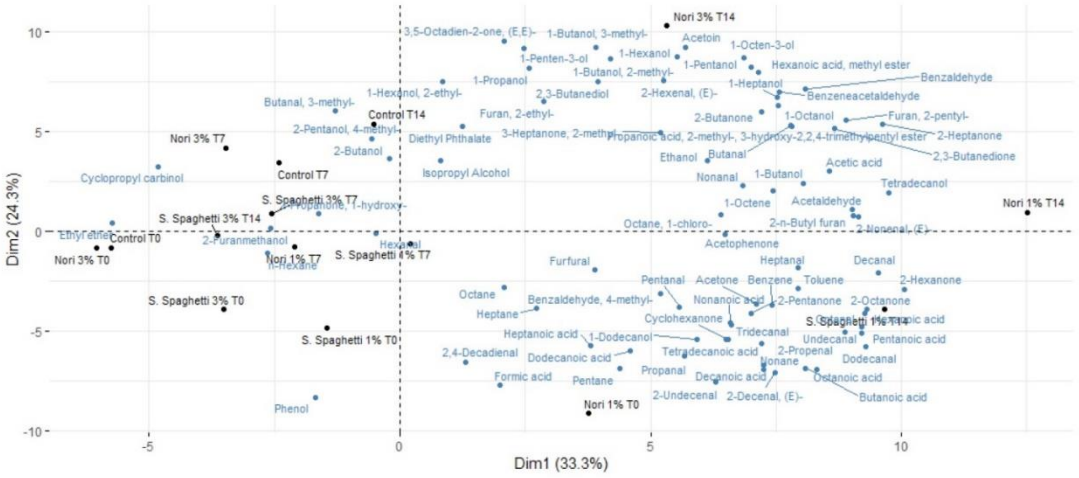


Figure 6.5. Principal Component Analysis (PCA) scores plot displaying the distribution of volatile compounds of beef and reformulated patties (1 % and 3 % seaweed) stored in refrigeration for 14 days in MAP, for the first two principal components (PC1 and PC2).

6.4.7. Gas chromatography-olfactometry

Characterization of aroma active compounds was conducted by HS-HiSorb GC-O on the beef and reformulated beef patties (Table 6.4). In total 78 aroma active volatiles were identified, including aldehydes (n=21), ketones (n=14), acids (n=11), alcohols (n=9), lactones (n=7), esters (n=6), furans (n=4), alkanes (n=3), ethers (n=2) and a phenol. Odorant compounds that have the same functional group tend to have similar odours (Table 6.4) (Genva et al., 2019). In general terms, aldehydes are associated with green odours such as grass cuttings or leaves, volatile fatty acids have a sour to rancid smell, esters have a fruity and floral smell, and lactones display a coconut or apricot character (Buettner, 2017). Indeed, some odorant molecules have the same functional group but different odours (i.e. some lactones) (Genva et al., 2019). Panellists perceived additionally 5 unidentified aroma active compounds, described as 'musty', 'potato', 'fruity, sweet', 'burnt' and 'bready' notes. The aroma of volatile compounds present at a concentration above its odour threshold but below its limit of detection by the mass spectrometer can be perceived by GC-O but cannot be identified (Vilar et al., 2021).

Some odour descriptors (partially) differed from the literature descriptors (Table 6.4). This may be explained by the factors that influence the perception of an odour. Aromatic VOC in higher concentrations activate a larger number of receptors. Hence, the number of activated receptors is linked both to the identity of an odorant as well as its concentration (Su et al., 2009). Another aspect that may have an impact is temporality; some aromatic VOC can cause a long-lasting response in some olfactory receptors and a shorter response in others (Su et al., 2009), thus in scenarios where aromas elute in rapid succession, such as in GC-O, some may not be perceived. Moreover, human olfaction perception is highly variable from one individual to another for both specific sensitivity and general olfactory acuity (Ferdenzi et al., 2017), due to olfactory perception diversity genetics, but also depending on culture, age, gender, and health status (Genva et al., 2019).

Table 6.4. Aroma-active compounds detected in the control and reformulated beef patties (3 % seaweed) by GC-O.

Compound[a]	CAS #	RI[b]	Odour intensity[c]			Odour descriptor	Literature ref.[d]	Odour type[d]	*P*-value
			C	S	N				
ACIDS									
Acetic acid	64-19-7	811	1.6	1.6	1.7	Vinegar / Sour / Acidic / Sharp	Sharp / pungent / sour / vinegar / acidic	Acidic	0.270
Butanoic acid	107-92-6	1003	2.3	1.5	2.5	Cheese / Fruity / Rancid	Sharp / acetic / cheese / butter / dairy-like / fruit	Cheesy	0.001*
Pentanoic acid	109-52-4	1096	1.9	2.3	2.5	Cheese / Rancid / Dairy / Fruity / Sweaty / Metallic / Potato / Oily	Acidic / sweaty / rancid / sharp / cheese / sour / milky / tobacco / fruity	Cheesy	0.630
Hexanoic acid	142-62-1	1206	1.6	2.0	nd	Fatty / Sweaty / Sweet / Bready	Sour / fatty / sweat / cheese	Fatty	0.215
Heptanoic acid	111-14-8	1304	1.4	1.7	2.8	Fruity / Pineapple / Fermented / Rancid / Cheesy	Rancid / sour / cheesy / sweat / waxy / fermented / pineapple / fruity	Cheesy	0.071
Hexanoic acid, 2-ethyl-	149-57-5	1338	nd	nd	2.7	Dump / Leafy / Green	n/a	Unknown	n/a
Octanoic acid	124-07-2	1388	1.3	2.2	2.0	Oily / Fatty / Rancid / Vegetable / Waxy	Fatty / waxy / rancid / oily / vegetable / cheesy	Fatty	0.621
Nonanoic acid	112-05-0	1454	1.8	1.3	nd	Waxy / Cheese / Dairy / Cultured	Waxy / dirty / cheese / cultured / dairy	Waxy	0.065
Decanoic acid	334-48-5	1511	2.8	1.8	1.0	Unpleasant / Sour / Citrus / Soapy / Floral	Unpleasant / rancid / sour / fatty / citrus	Fatty	1.694
Undecanoic acid	112-37-8	1573	1.7	nd	nd	Waxy / Fatty / Creamy	Waxy / creamy / cheese / fatty / coconut	Waxy	n/a
Dodecanoic acid	143-07-7	1651	0.5	1.7	1.2	Fatty / Oily / Cucumber / Fresh	Mild / fatty / coconut / bay / oil	Fatty	0.194
ALCOHOLS									

2-Pentanol, 4-methyl-	108-11-2	920	1.6	1.2	nd	Alcoholic / Pungent / Floral / Green	Pungent / alcoholic	Pungent	0.485
1-Pentanol	71-41-0	934	1.9	2.2	1.8	Rubber / Pungent / Yeasty / Fermented / Winey / Sweet / Musty	Pungent / fermented / bready / yeasty / fusel / winey / solvent-like / oily / sweet / balsamic	Fermented	0.034*
2,3-Butanediol	513-85-9	996	2.2	nd	1.8	Fruity / Sweet	Fruity / creamy / buttery	Creamy	0.032*
1-Hexanol	111-27-3	1043	2.1	1.8	2.1	Green / Alcoholic / Fusel / Ethereal / Pungent / Herbal	Ethereal / fusel / oil / fruity / alcoholic / sweet / green / pungent	Herbal	0.274
1-Octen-3-ol	3391-86-4	1157	2.6	2.4	2.3	Mushroom / Earthy / Vegetative / Metallic	Mushroom / earthy / green / oily / fungal / raw chicken / vegetative	Earthy	0.111
1-Hexanol, 2-ethyl-	104-76-7	1212	nd	1.3	nd	Fatty / Sweaty / Pungent	Citrus / fresh / floral / oily / sweet	Citrus	n/a
1-Octanol	111-87-5	1259	2.5	2.4	2.7	Mushroom / Floral / Green / Orange / Waxy / Fatty / Rose / Aldehydic / Earthy	Waxy / green / orange / aldehydic / rose / mushroom / citrus / floral / sweet / fatty / coconut	Waxy	0.071
Phenylethyl Alcohol	60-12-8	1335	nd	1.4	nd	Floral / Rose / Fresh / Sweet	Floral / rose / dried rose / rose water / sweet / fresh / bready / honey	Floral	n/a
1-Dodecanol	112-53-8	1561	2.7	1.2	1.7	Soapy / Waxy / Honey / Nutty / Creamy	Earthy / soapy / waxy / fatty / honey / coconut	Waxy	3.083
ALDEHYDES									
Butanal	123-72-8	731	nd	nd	2.3	Malty / Cocoa	Pungent / cocoa / musty / green / malty / bready	Chocolate	n/a
Butanal, 3-methyl-	590-86-3	797	2.4	2.8	2.8	Chocolate / Aldehydic / Fatty / Ethereal / Fruity	Ethereal / aldehydic / chocolate / peach / fatty	Aldehydic	0.093
Pentanal	110-62-3	840	nd	1.6	1.5	Fruity / Floral	Fermented / bready / fruity / nutty / berry / diffusive	Fermented	0.132
Hexanal	66-25-1	952	2.5	1.9	2.3	Grass / Green / Leafy / Fresh	Fresh / green / fatty / aldehydic / grass / leafy / fruity	Green	0.218

							/ sweaty / vegetative / clean / woody		
Furfural	98-01-1	1016	2.2	2.3	nd	Almond / Woody / Fragrant / Baked / Nutty / Cheese	Sweet / woody / almond / fragrant / baked / bread	Bready	0.322
2-Hexenal	505-57-7	1025	nd	1.0	1.0	Almond / Sweet	Sweet / almond / fruity / green / leafy / apple / plum / vegetable	Green	0.500
Heptanal	111-71-7	1064	3.2	2.8	3.1	Green / Herbal / Fresh / Wine-lee	Fresh / aldehydic / fatty / green / herbal / wine-lee / ozone	Green	0.004*
Benzaldehyde	100-52-7	1150	2.9	3.0	2.8	Almond / Bitter / Strong	Strong / sharp / sweet / bitter / almond / cherry	Fruity	0.234
Octanal	124-13-0	1175	2.4	2.5	2.9	Citrus / Orange peel / Fresh / Waxy	Aldehydic / waxy / citrus / orange peel / green / herbal / fresh / fatty	Aldehydic	0.031*
Benzeneacetaldehyde	122-78-1	1246	nd	2.2	2.3	Floral / Rose / Fresh / Cucumber / Leafy	Green / sweet / floral / hyacinth / clover / honey / cocoa / rose / powdery / fermented / chocolate	Green	0.072
2-Octenal, (E)-	2548-87-0	1252	2.2	2.5	2.2	Herbal / Fresh / Green / Cucumber / Leafy / Fatty / Floral / Earthy	Fresh / cucumber / fatty / green / herbal / banana / waxy / green / leaf	Fatty	0.041*
Nonanal	124-19-6	1287	2.8	2.8	2.5	Citrus / Orange peel / Lemon peel / Fresh / Waxy / Rose / Aldehydic / Soapy	Waxy / aldehydic / rose / fresh / orris / orange peel / fatty / peely / citrus / green / lemon peel / cucumber	Aldehydic	0.058
2-Nonenal, (E)-	18829-56-6	1356	3.1	2.6	3.1	Cucumber / Green / Citrus / Fatty	Fatty / green / cucumber / aldehydic / citrus	Fatty	<0.001*
Decanal	112-31-2	1373	1.5	1.4	2.3	Orange peel / Citrus / Floral /Sweet	Sweet / aldehydic / waxy / orange peel / citrus / floral	Aldehydic	0.804
2-Decenal, (E)-	3913-81-3	1433	1.6	1.6	2.1	Earthy / Green / Waxy / Fatty / Cilantro / Leafy	Waxy / fatty / earthy / green / cilantro / mushroom /	Fatty	0.030*

Undecanal	112-44-7	1453	nd	nd	1.8	Waxy / Soapy / Citrus / Fresh	aldehydic / fried chicken / fat / tallow / coriander / pork fat Waxy / soapy / floral / aldehydic / citrus / green / fatty / fresh / laundry	Aldehydic	n/a
2,4-Decadienal, (E,E)-	25152-84-5	1474	nd	1.9	1.4	Cucumber / Oily	Oily / cucumber / melon / citrus / pumpkin / nut / meat	Fatty	0.182
2,4-Decadienal	2363-88-4	1474	1.8	nd	nd	Green / Citrus / Orange / Fatty / Oily / Cucumber / Creamy / Waxy / Sweet / Coconut	Orange / sweet / fresh / citrus / fatty / green / oily / chicken skin-like	Fatty	n/a
2-Undecenal	2463-77-6	1496	0.5	nd	1.8	Fruity / Herbal	Fresh / fruity / orange peel	Fruity	0.107
Dodecanal	112-54-9	1515	1.3	nd	nd	Soapy / Waxy	Soapy / waxy / aldehydic / citrus / green / floral	Aldehydic	n/a
2-Dodecenal, (E)-	20407-84-5	1568	nd	nd	1.7	Waxy / Metallic	Citrus / metallic / mandarin / orange / waxy / aldehydic	Herbal	n/a
ALKANES									
Heptane	142-82-5	805	1.5	nd	nd	Sweet	Sweet / ethereal	Other	n/a
Nonane	111-84-2	1018	nd	nd	2.4	Cheese / Bready / Fermented / Green	Gasoline	Other	n/a
1-Octene	111-87-5	905	nd	nd	1.6	Green / Floral / Sweet	Gasoline	Other	n/a
ESTERS									
Isovaleric acid, methyl ester	556-24-1	915	nd	2.0	nd	Fruity / Sweet	Strong / apple / fruity / pineapple	Fruity	n/a
Hexanoic acid, methyl ester	106-70-7	1075	2.3	1.4	nd	Fruity / Pineapple / Ethereal	Ethereal / fruity / pineapple / apricot / strawberry / tropical / banana / bacon	Fruity	1.307
Isopropyl isovalerate	32665-23-9	1088	3.0	2.7	3.0	Bread crust / Woody	Apple / pineapple / fruity / winey	Fruity	0.023*
Heptanoic acid, methyl ester	106-73-0	1183	1.4	1.8	2.3	Waxy / Floral / Fresh / Soapy	Sweet / fruit / green / orris / waxy / floral / berry	Fruity	0.404
Dodecanoic acid, methyl ester	111-82-0	1580	nd	1.3	0.8	Waxy / Soapy / Petal	Waxy / soapy / creamy / coconut / mushroom	Waxy	0.042*

Myristic acid, methyl ester	124-10-7	1753	nd	nd	1.2	Waxy / Petal	Fatty / waxy / petal	Waxy	n/a
ETHERS									
Ethyl ether	60-29-7	631	1.3	nd	1.6	Ethereal / Solvent	Ethereal	Other	0.035*
Ethanol, 2-phenoxy-	122-99-6	1426	nd	1.8	nd	Waxy / Fresh / Toasted	Rose / balsam / cinnamyl	Floral	n/a
FURANS									
Furan, 2-butyl-	4466-24-4	1029	nd	1.2	1.2	Mild / Fruity / Wine / Green / Grass / Herbal	Mild / fruity / wine / sweet / spicy	Spicy	0.042*
Furan, 2-pentyl-	3777-69-3	1138	2.7	2.6	2.3	Beany / Earthy / Metallic / Vegetable / Green / Fruity	Fruity / green / earthy / beany / vegetable / metallic	Fruity	0.040*
Furan, 2-hexyl-	3777-70-6	1249	nd	nd	2.4	Fatty / Sour / Sweat	n/a	Unknown	n/a
Furan, 2-heptyl-	3777-71-7	1351	2.6	2.4	2.8	Green / Fatty / Oily / Nutty / Herbal / Cucumber / Fresh	Green / fatty / lactonic / oily / roasted / nutty / dairy	Green	0.010*
KETONES									
2,3-Butanedione	431-03-8	736	2.8	2.7	2.8	Butter / Caramel	Strong / butter / sweet / creamy / pungent / caramel	Buttery	0.164
2-Pentanone	107-87-9	835	nd	2.2	nd	Fruity	Sweet / fruity / ethereal / wine / banana / woody / fermented	Fruity	n/a
Acetoin	513-86-0	891	2.0	1.7	1.9	Milky / Buttery / Dairy / Fatty	Sweet / buttery / creamy / dairy / milky / fatty	Buttery	1.654
2-Hexanone	591-78-6	946	nd	nd	1.5	Buttery / Fruity	Fruity / fungal / meaty / buttery	Fruity	n/a
2-Heptanone	110-43-0	1056	2.2	2.2	2.1	Cheese / Woody / Ketonic / Spicy / Fruity / Creamy / Sweet	Fruity / spicy / sweet / herbal / coconut / woody / cheese / fruity / ketonic / green / banana / creamy	Cheesy	0.188
3-Heptanone, 2-methyl-	13019-20-0	1100	2.1	1.9	1.9	Green / Fruity / Leafy	Fruity / green / leafy	Fruity	0.231
2-Octanone	111-13-7	1166	nd	nd	2.1	Herbal / Weedy / Earthy	Earthy / weedy / natural / woody / herbal / musty /	Earthy	n/a

							ketonic / bleu and parmesan cheese-like		
5-Hepten-2-one, 6-methyl-	110-93-0	1163	nd	2.5	nd	Earthy / Green / Herbal / Pungent	Citrus / green / musty / lemongrass / apple / ketonic / creamy / cheesy / banana	Citrus	n/a
Acetophenone	98-86-2	1271	1.6	2.3	2.0	Sweet / Mimosa / Cherry / Nutty / Marzipan / Almond / Pungent	Sweet / pungent / hawthorn / mimosa / almond / acacia / chemical / cherry / marzipan / coumarinic / nutty / heliotrope / vanilla	Floral	0.670
2-Nonanone	821-55-6	1280	nd	nd	1.6	Herbal / Fresh / Fruity / Sweet	Fresh / sweet / green / weedy / earthy / herbal / fruity / waxy / soapy / cheese / herbaceous / coconut	Fruity	n/a
3,5-Octadien-2-one, (E,E)-	38284-27-4	1296	1.8	nd	nd	Citrus / Floral	Fruity / green / grassy	Fruity	n/a
2-Decanone	693-54-9	1373	1.8	nd	1.8	Floral / Orange / Fatty	Orange / floral / fatty / peach	Floral	0.042*
2-Undecanone	112-12-9	1445	nd	1.7	1.9	Waxy / Fruity / Floral / Fatty / Grassy / Herbal	Waxy / fruity / creamy / fatty / orris / floral	Fruity	0.132
2-Pentadecanone	2345-28-0	1748	nd	1.8	nd	Waxy / Sweet	Fresh / jasmin / celery	Floral	n/a
LACTONES									
γ-Hexalactone	695-06-7	1302	nd	1.8	1.5	Herbal / Tobacco / Sweet / Coconut	Herbal / coconut / sweet / coumarin / tobacco / creamy / lactonic / green	Tonka bean	0.032*
δ-Valerolactone	542-28-9	1316	2.2	nd	nd	Metallic / Musty / Herbal	N/A	Unknown	n/a
γ-Heptalactone	105-21-5	1383	nd	nd	1.8	Coconut	Sweet / coconut / nutty / caramel / tonka / hay / coumarin / lactonic / creamy / powdery	Coconut	n/a
γ-Octalactone	104-50-7	1466	nd	2.3	1.8	Creamy / Fatty / Waxy / Sweet / Coconut / Dairy	Sweet / coconut / waxy / creamy / tonka / dairy / fatty /	Coconut	0.515

							milky / soapy / coumarin / fruity / peach / apricot		
γ-Nonalactone	104-61-0	1535	nd	2.0	nd	Coconut / Sweet / Fatty	Coconut / creamy / waxy / sweet / buttery / oily / fatty	Coconut	n/a
γ-Dodecalactone	2305-05-7.	1816	nd	nd	2.0	Fruity / Metallic	Fatty / peach / sweet / metallic / fruity	Fruity	n/a
δ-Dodecalactone	713-95-1	1880	nd	1.7	nd	Peach / Metallic	Fresh / sweet / metallic / peach / oily / coconut / buttery / fatty / creamy / dairy / fruity / peach / apricot / milky	Tropical	n/a
PHENOLS									
Phenol	108-95-2	1243	1.9	nd	nd	Rubber	Phenolic / plastic / rubber	Phenolic	n/a
SUM OF INTENSITY			96.2	106.7	117.1				

RI, retention index; C, control; S, Sea Spaghetti; N, Nori; n/a, not available/applicable; nd, not detected; *P <0.05 between groups; [a] Identification based on NIST mass spectral database, RI values from the literature and an in-house library created using authentic compounds with target and qualifier ions and linear RI for each compound; [b] Retention index calculated from GC-O results on a DB-624 UI column; [c] Mean odour intensity for each beef patties (3 % seaweed) evaluated by sniffers. From 1 = low to 4 = high; [d] Odour descriptors found in the literature [http://www.thegoodscentscompany.com/index.html].

The samples were classified by 'acidic', 'aldehydic', 'bready', 'buttery', 'cheesy', 'chocolate', 'citrus', 'coconut', 'creamy', 'earthy', 'fatty', 'fermented', 'floral', 'fruity', 'green', 'herbal', 'phenolic', 'pungent', 'spicy', 'tonka bean', 'tropical' and 'waxy' odour types according to descriptors provided by The Good Scents Company (The Good Scents Company, n.d.) (Table 4). In general terms, control samples were mostly characterized by 'green, earthy, buttery' aroma; patties containing Sea Spaghetti by 'earthy, aldehydic, bready' notes and those containing Nori by 'aldehydic, cheesy, buttery, chocolate' aromas (Fig. 6.6).

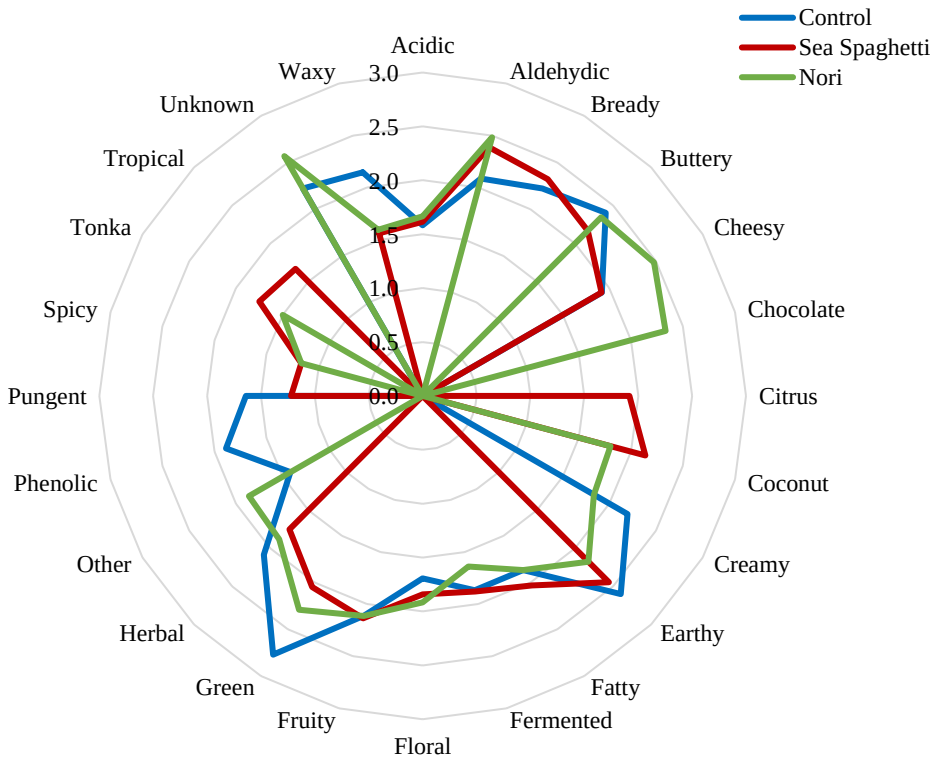


Figure 6.6. Radar diagram of the odour type of raw beef patties (control) and reformulated patties with seaweeds (Sea Spaghetti - *H. elongata*, and Nori - *P. umbilicalis*). Average scores are shown according to quantitative descriptive odour intensities evaluated by five panellists (Table 6.4).

Nori patties had the strongest odour of the three samples, as the sum of all intensity scores was the highest at 117.1, where the control samples had the lowest score, 96.2 (Table 6.4). This is presumably due to the lack of seaweed in these

samples, as higher intensity scores were obtained with higher percentage of seaweed addition (1 % < 3 % seaweed), thus more VOC and more abundance. No statistically significant differences (P >0.05) were observed across time within each treatment (Table S-6.2).

1-Octen-3-ol (mushroom, earthy, vegetative, metallic), heptanal (green, herbal, fresh, wine-lee) and isopropyl isovalerate (bread crust, woody) had the highest intensity scores across all samples, thus indicating that these compounds are likely important VOC in these beef patties and are independent of seaweed addition. Control samples were characterized by decanoic acid (unpleasant, sour, citrus, floral, soapy), 1-dodecanol (soapy, waxy, honey, nutty, creamy), benzaldehyde (almond, bitter, strong), nonanal (citrus, orange/lemon peel, fresh, waxy, rose, aldehydic, soapy), 2-pentylfuran (beany, earthy, metallic, vegetable, green, fruity), 2-heptanone (cheese, woody, ketonic, spicy, fruity, creamy, sweet), δ-valerolactone (metallic, musty, herbal) and phenol (rubber). Sea Spaghetti samples were dominated by pentanoic acid (fruity, sweaty, rancid, cheese, dairy, metallic, potato, oily), octanoic acid (oily, fatty, rancid, vegetable, waxy), 1-octanol (mushroom, floral, green, orange, waxy, fatty, rose, aldehydic, earthy), 3-methylbutanal (chocolate, aldehydic, fatty, ethereal, fruity), benzaldehyde (almond, bitter, strong), nonanal (citrus, orange/lemon peel, fresh, waxy, rose, aldehydic, soapy), 2-pentylfuran (beany, earthy, metallic, vegetable, green, fruity), 2,3-butanedione (butter, caramel), 6-methyl-5-hepten-2-one (green, herbal, earthy, pungent) and γ-octalactone (creamy, fatty, waxy, sweet, coconut, dairy). Butanoic acid (cheese, fruity, rancid), heptanoic acid (fruity, pineapple, fermented, rancid, cheesy), 1-octanol (mushroom, floral, green, orange, waxy, fatty, rose, aldehydic, earthy), octanal (citrus, orange peel, fresh, waxy), (E)-2-nonenal (cucumber, green, citrus, fatty), 2-heptylfuran (green, fatty, oily, nutty, herbal, cucumber, fresh), 2-heptanone (cheese, woody, ketonic, spicy, fruity, creamy, sweet), 2-octanone (herbal, weedy, earthy) and γ-dodecalactone (fruity, metallic) dominated the Nori samples. The main statistically significantly different odour-active compounds between all samples were butanoic acid and heptanoic acid, and octanal between the control and Nori samples. All three compounds increased over time, except for heptanoic acid which decreased in control samples.

6.5 Conclusion

In this study, we have demonstrated that HiSorb can be used in conjunction with TD GC-MS and GC-O analysis to provide valuable information on the volatile aroma profile of raw beef patties and in reformulated patties containing seaweed.

Inclusion of seaweed did not impact gas composition within the MAP, nor did the inclusion of seaweed impact on the fat or moisture of the samples. Overall, the microbiological results highlighted satisfactory ACC and ß-glucuronidase + *E. coli* levels through storage, whereas *Enterobacteriaceae* only reached unsatisfactory levels by day 14. The lightness of the samples increased with increasing storage time, whereas redness decreased. The seaweed impacted the increment of blue and loss of yellow in some samples. Even though the inclusion of the seaweed increased antioxidant activity it was not sufficient to significantly impact lipid oxidation over storage in the beef patties. The incorporation of seaweed increased the VOC content and the VOC changed over storage, with most chemical classes increasing in all samples. The odour intensity of the samples was in the order of Nori > Sea Spaghetti > Control and the inclusion of seaweed impacted the odour profile of the samples.

This study has highlighted the potential of seaweed addition in the production of beef patties, but that much more work is required in relation to selection of seaweed species, processing parameters, extract type, dose rate to determine if it can be successfully used to reduce lipid oxidation and decolourisation maintaining quality over storage without adversely impacting on sensory appeal.

6.6 Limitations

Sorptive extraction works on the principle of absorption, which is an equilibrium process. The maximum achievable extraction efficiency for a particular analyte therefore depends upon its particular characteristics, and it may not be possible to achieve 100 % recovery in all cases.

6.7 References

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Chapter 7

Chapter 7. General discussion, conclusions, and future possibilities

7.1. Background

The influence of diet on the risk of non-communicable diseases has been well proven in the last few decades (Machado et al., 2022). A promising approach to better health care would be to produce a healthier food supply, which could be improved by manufacturing functional foods with natural ingredients that have healthier nutritional profiles than conventional products (Decker and Park, 2010).

Meats have great potential for delivering important nutrients such as fatty acids, minerals, dietary fiber, antioxidants and bioactive peptides into the diet (Decker and Park, 2010). However, it is believed that higher red meat and processed meat consumption increases the risk of disease (Zhang et al., 2021), due to components such as sodium, saturated fat, and additives (Zheng et al., 2019), causing many health-conscious people to limit their intake.

Considerable efforts have been made to optimize meat and meat products composition to help consumers adapt their diet to nutrient intake goals. Meat-based functional foods have been formulated both by reducing the concentrations of some unhealthy compounds (e.g., fat and sodium) and by promoting the presence of healthy compounds in functional ingredients (e.g., algae) (Jiménez-Colmenero et al., 2010). Emphasizing these activities is one possible approach to improving the health image of meat products and by continuing to develop more functional meat products (Arihara, 2006).

This research has highlighted the need for further studies to elucidate the changes in sensory perception of processed meat products that occur upon reformulation, and to understand the role of added natural ingredients (seaweed), to aid in the development of suitable reformulated products that meet consumer demands/expectations. This Doctoral thesis carried out novel research required to further understand the effects of seaweed addition as a replacer of salt content on the aromatic and sensory properties of meat and processed meat products, and to

evaluate informed innovative formulations on the shelf life of selected meat products.

7.2. Scientific and industrial impact

The meat sector in Ireland is one of the most important indigenous industries in the national economy, supporting in excess of 120,000 individual farmers and generating total sales of more than €4.5 bn, with 2020 exports of approximately €3 bn (Meat Industry Ireland - Ibec, 2022). However, the meat industry may be jeopardised as a high consumption of processed meat, such as sausages, salami, ham, and bacon, has been consistently associated with a higher risk of hypertension, diabetes (Thao et al., 2022), and heart disease (Al-Shaar et al., 2020).

People are becoming increasingly aware of food quality and the health benefits associated with different foods. Therefore, it is necessary to develop novel functional foods to meet the demand for healthy food products (Baker et al., 2022). This demand from modern consumers is pushing the food industry to decrease or even eliminate the use of synthetic additives in manufacturing; thus, numerous research and technological applications have switched to focus on natural counterparts (Granato et al., 2020). Focusing on the meat sector, the consumer demands for healthier meat and meat products with a reduced level of fat, and cholesterol, decreased contents of sodium chloride and nitrite, improved composition of fatty acid profile and incorporated health-enhancing ingredients are rapidly increasing worldwide (Zhang et al., 2010).

Ireland, with an extensive coast of over 3000 km, has a long history of seaweed use that continues up to the present day. In particular, Ireland's Atlantic seaboard is ideal for the settlement of seaweeds and consequently is particularly rich in seaweed resources (Morrissey et al., 2001). Marine foods and their ingredients including seaweeds, macroalgae and microalgae can be added to different food products such as meat-based products to make them more functional (Roohinejad et al., 2017). In addition to the vast range of functional properties such as nutritional, physicochemical and textural properties that seaweeds impart to food products,

many studies showed health benefits either when consumed directly or after minor pre-processing (Lomartire et al., 2021). Besides, the potential of using seaweed powder and extracts against lipid oxidation in foods has been widely studied (Moroney et al., 2013).

However, the adoption of functional foods by consumers is a difficult and slow process that depends on several different variables (e.g. social, economic, geographical, cultural, and ethnic backgrounds). Understanding consumers' reactions to novel foods is essential since their scepticism and uncertainty may affect their acceptance (Baker et al., 2022). The effect of taste on consumer acceptance has received considerable attention in previous studies. Flavour or expected flavour strongly influences consumers' functional food choices (Jung et al., 2020). For example, a study conducted by Narayana et al. (2020) found that flavour was one of the most important motives for consuming functional foods among consumers. Given that several studies (Moons et al., 2018; Temesi et al., 2019; Topolska et al., 2021) have shown that customers are hesitant to sacrifice flavour in favour of health advantages, the effect of flavour may frequently outweigh the influence of health benefits (Papoutsi et al., 2019; Wortmann et al., 2018). As Verbeke (2006) argued, it is highly risky to assume that consumers would accept functional foods that are not flavoursome. Hence, sensory studies on product odour and taste should be conducted early in the research and development process, as per revealed, sensory preferences are likely a critical driver influencing functional food consumption.

7.3. Understanding shelf life

Several factors influence lipid oxidation in meat products, but the main two are fat content and fatty acid composition because fatty acids are the substrate of the oxidation processes. Knowing that oxidative processes are multiple chemical reactions, like any other chemical reaction, lipid oxidation is favoured as both a time and temperature increase (Domínguez et al., 2019). As expected, the contact between the substrates and the oxidation catalyst is favoured by the temperature.

However, not only the temperature value is decisive, but the possibility also that radicals cause damage to lipids increases with storage time (Domínguez et al., 2019).

Modern packaging methods offer advantages over conventional techniques to preserve the product. It should be noted that the gas composition of the atmosphere surrounding the product is essential for the development of the oxidative processes. From the point of view of lipid oxidation, considering that oxygen acts as the main component of the propagation phase through its ability to react with alkyl radicals to form peroxy radicals and also as a source of reactive oxygen species, it seems evident that reducing its concentration in the package is a good strategy to limit the oxidation of meat (Amaral et al., 2018). However, it is not always possible to eliminate oxygen. The clearest case is in fresh meat, especially red meat, which must be packaged in a rich O_2 atmosphere to maintain the desirable red colour. The main drawback to using high concentrations of O_2 is the development of rancidity that can even occur without appreciable deterioration of the colour. Therefore, taking into account the product to be packaged (fresh meat or meat product), the correct choice of the packaging/packaging material, the technology used to minimise oxidation and the presentation format are all of vital importance in minimizing quality loss whilst still ensuring consumer appeal.

The wide variety of factors that influence oxidation, different compositions of meat and meat products, as well as the complexity of reactions and interactions during lipid oxidation make it practically impossible to develop a unique technique to measure the degree of oxidation. However, taking into account the importance that oxidation has on product quality, it is necessary to measure the level of oxidation in order to establish strategies to minimize quality losses. For this reason, there are a variety of analytical techniques that help in the identification and quantification of changes in reagents and products of lipid oxidation, such as peroxide values and thiobarbituric acid reactive substances (TBARS) but these often fail to accurately capture the extent of lipid oxidation issues in products and do not provide sufficient information as to the source of adverse odours, or potential lipid oxidation stability of a product. Therefore, identifying and understanding the concentrations of specific volatiles involved in lipid oxidation is critical in maximising shelf-life, maintaining quality and consumer appeal. Although methods for identifying and

measuring/monitoring lipid oxidation volatiles by gas chromatography-mass spectrometry (GC-MS) or gas chromatography (GC)-flame ionization detector (FID) in meat products have been studied, new extraction techniques such as high capacity sorptive extraction have been developed that could provide more information once optimised and validated. To better understand the impact of specific key volatiles on product quality GC-MS techniques in association with gas chromatography-olfactometry (GC-O) and/or sensory analysis can provide a much more in-depth understanding, especially for reactions such as lipid oxidation that could maximise shelf life and product quality of meat and meat products.

7.4. Thesis highlights

Chapter 1

- Beef and pork are reservoirs of volatile organic compounds (VOC) that contribute to the development of complex flavours during cooking.
- It is the use of olfactometry methodologies and mass spectrometry (MS) that contribute to more accurate information about the overall aroma of the food.
- The key volatiles of cooked beef include octanal, nonanal, (E,E)-2,4-decadienal, methanethiol, methional, 2-furfurylthiol, 2-metyl-3-furanthiol, 3-mercapto-2-pentanone, and 4-hydroxy-2,5-dimethyl-3-(2H)-furanone.
- The key volatiles of pork flavour are hexanal, 1-nonanal, 3-methyl-1-butanol, 1-penten-3-one, 2-pentyl-furan, N-morpholinomethyl-isopropyl-sulphide, and methyl butyrate.
- The impact of sulphur-containing compounds is significantly important in beef and pork aroma.

Chapter 2

- More than 200 different volatile compounds, belonging to 13 or more chemical groups, were present in *Himanthalia elongata*, *Laminaria* spp., *Undaria pinnatifida*, *Laminaria ochroleuca*, *Porphyra umbilicalis* and *Palmaria palmata*.

- The major volatile compounds found in seaweeds, in decreasing order, were hydrocarbons, ketones, aldehydes, alcohols, halogen or sulphur-containing compounds, acids, esters, furans, and phenols.
- The impact of volatile compounds on sensory perception is dependent upon abundance and odour threshold.
- Extended work needs to be done related to the volatile compounds of seaweeds in connection with their sensory characteristics and their influence on the acceptability of new seaweed-containing products.

Chapter 3

- The volatile profiles of *Himanthalia elongata*, *Undaria pinnatifida*, *Porphyra umbilicalis* and *Palmaria palmata* were described, including those of *Alaria esculenta* and *L. japonica* fucoxanthin extract for the first time.
- Volatile compounds differed intensely in the five varieties of dried seaweed and powder extract.
- *P. palmata* had the strongest odour, whereas *L. japonica* fucoxanthin extract had the lowest odour of all samples.
- Green seaweed, *H. elongata* and *U. pinnatifida*, were characterized by 'fresh, marine, fishy' and 'grassy, herbal, floral, vegetables' aroma which have been attributed to (E,Z)-2,6-nonadienal and sulcatone. *A. esculenta* was characterized by 'grassy, herbal', 'fruity' and 'mushroom earthy' notes which were attributed to 1-octen-3-ol, 3-heptanone and benzaldehyde.
- Red seaweed, *P. palmata* was characterized by 'fresh, marine, fishy' and 'oily' aromas which have been attributed to 2,6-dimethyl-pyrazine and 3-heptanone. *P. umbilicalis* was characterized by 'mushroom, earthy, damp' and 'rancid, oily, fatty' notes attributed to 3 unidentified compounds.
- *L. japonica* fucoxanthin extract was characterized by 'nutty, toasted' and 'fatty, rancid' aromas which were attributed to 2-methyl-nonane and 2-furanmethanol.
- 1-Octen-3-ol (mushroom, mineral, hay) and heptanal (herbal, grassy) were common and appeared signification in all samples but differed in their intensities.

- The characterization of volatiles in seaweeds may help to develop new products that are more acceptable to consumers.

Chapter 4

- The inclusion of seaweed and reduction of salt and fat impacted the sensory and physiochemical properties of frankfurters.
- Significant differences in colour, liking of appearance, aroma, flavour and texture attributes were evident between the samples.
- The overall acceptability of reformulated frankfurters containing seaweed was greatly influenced by the type of added seaweed.
- Reformulated frankfurters containing *H. elongata* were the most accepted.
- Inclusion of seaweed greatly influenced the abundance of volatiles compounds.
- The addition of *U. pinnatifida*, *P. umbilicalis*, *P. palmata* and especially *H. elongata* may have the potential to improve the nutritional quality through mainly salt reduction.

Chapter 5

- A novel lipid oxidation method for the quantification of targeted lipid oxidation volatile compounds was developed (high capacity sorptive extraction (HiSorb) thermal desorption (TD) GC-MS).
- Nine compounds derived from lipid oxidation were quantified by HiSorb TD GC-MS.
- A central composite rotatable design was used to optimize HiSorb extraction parameters.
- Response surface methodology assessed the effects of extraction parameters.
- Method validation included limits of detection (LOD), limits of quantification (LOQ), and precision.
- The method was most sensitive for 1-hexanol and 2-heptanone as lipid oxidation compounds biomarkers.
- The overall lipid oxidation trend by the HiSorb TD GC-MS was similar to TBARS but provides significantly more specific information.
- The validated method may be beneficial in terms of amelioration of product shelf life stability.

- Lipid oxidation, associated with a rancid odour, leads to the deterioration of food quality.
- Significant colour differences were seen between day 0 and days 7 and 14 of storage.
- TBARS content increased throughout the storage period, observing lower levels of lipid oxidation in 3 % Sea Spaghetti and Nori beef patties.
- Sea Spaghetti had higher total phenol content (TPC) than Nori.
- The storage time influenced the abundance of VOC discriminating samples: 82 VOC in the reformulated beef patties vs. 64 VOC in the control.
- 78 aroma active volatiles were identified by HiSorb TD GC-MS/O.
- Nori patties had the strongest odour.
- Control samples were characterized by 'green, earthy, buttery' aroma; Sea Spaghetti-containing patties by 'earthy, aldehydic, bready' notes and Nori-containing patties by 'aldehydic, cheesy, buttery, chocolate' aroma.
- The incorporation of seaweeds altered the volatile aromatic profile of the patties.

7.5. Conclusions

This thesis outlines different approaches taken to further investigate the application of natural ingredients in the reformulation of processed meat products.

It is possible to have a more in-depth understanding of the flavour and aroma formation that affect sensory perception by thoroughly characterizing the volatile aroma profile of the product, both before and after reformulation. This information can be used to make informed formulation changes that can improve consumer acceptability and even enhance the shelf life of the product. It is important to maintain the proper balance of volatile aromatic compounds to stop the development of undesirable off-odours, which are mostly brought on by lipid oxidation in meat products. On selection of suitable edible seaweed as salt replacers and lipid oxidation inhibitors, the processing of seaweed before addition, the

optimization of dose rates, and the selection of less intense seaweed-flavour algae are necessary before the reformulated products are likely to be accepted by consumers unfamiliar with seaweeds in their diet. Choosing adequate raw ingredients for optimum reformulation requires consideration of their intrinsic sensory characteristics. This doctoral thesis has highlighted the potential of combining multiple sensory techniques, volatile profiling and olfactometry to better understand the effects of formulation changes in processed meat products with edible seaweed on sensory perception and advance our general understanding of flavour development in these products. The results are therefore of interest to the meat industry, manufacturers, marketers, and consumers alike.

7.6. Future recommendations

7.6.1. Overcoming limitations

Gas chromatography-Olfactometry alone does not allow a conclusion on the contribution of a single compound to the overall aroma, as mentioned in Chapter 3. Evaluating different extraction techniques and sorbent phases will enable more VOC to be extracted and perceived, thus providing more information on the volatile composition of seaweeds. Chemometrically combining GC-O data with descriptive sensory data, may provide a better understanding of aroma/sensory associations. Lastly, utilising sensory omission studies (de Jong et al., 2019), where individual odour-active VOC are removed from a sample and the change in aroma balance is assessed. Removal of a single VOC can alter the overall profile and thus give a greater understanding of the relevance of that VOC to the quality of the final product. .

In chapter 3, it was also highlighted how different companies using different harvesting and post-harvesting strategies can influence the volatile profile of the seaweeds, which also applies to the methods used to prepare seaweed extracts. A better understanding of how different food processes influence the volatiles profile of seaweed is essential, as it would be a powerful tool to tailor sensory properties and improve the final consumer acceptance of algae in food products. Ideally a

standard drying procedure should be developed to maximise the quality of seaweed, to minimise losses of important components/nutritional quality, but also maximise shelf life and for safe use in the food or beverage industry. The same would apply to seaweed extracts. As the industry develops this is likely to evolve due to greater regulation depending upon the application.

Sorptive extraction (used in Chapter 5) works on the principle of absorption, which is an equilibrium process. The maximum achievable extraction efficiency for a particular analyte depends upon its characteristics (volatility, polarity, vapour pressure and molecular weight) and the composition of the sample, and therefore it may not be possible to achieve 100 % recovery in all cases due to the range of factors involved that may influence the process. The application of multifunctional volatile extraction techniques, with enrichment capacity such as available on the Centri Sample Automation system (Markes International Ltd.) will enable more representative volatile profiling of samples. This, in addition to advances in comprehensive or two-dimensional gas (GCxGC) chromatography together with high resolution mass spectrometry will significantly improve sensitivity and capability of volatile analysis, providing much more information than the techniques used in this thesis.

7.6.2. Future work

This thesis has covered the optimization of frankfurters and beef patties formulations through the incorporation of edible seaweed as a salt reduction clean label approach. Different reformulated processed meat products (e.g., pudding) could be used, and the determination of other or a combination of clean-label sodium replacers like seaweed that could produce a desirable reduced in salt formula with optimum aroma compound formation and still be palatable would complement this work.

VOC were analysed to understand the influence of matrix changes on the character aroma compounds and sensory perception. However, the identification of anchor non-volatile compounds (sugars and nucleotides, fatty acids) for key sensory attributes

would reinforce the study. The VOC were analysed using GC-MS, but the use of two-dimensional GC with high resolution MS would elucidate more VOC likely influencing aroma perception, and in tandem with olfactory and sensory analysis would obtain a complete volatile profile.

Modified atmosphere packaging (MAP) was the preferred type of packaging for preserving the quality of the beef patties and for extending their shelf life and stability. However, the differences in the various meat products require the packaging concept to be designed in individual ways. The interaction of various packaging systems and materials on fresh meat and the comparison of different packaging variants would increase value of the studies.

Developing products that are preferred overall, or liked as well as, or better, on average, compared to a standard or a competitor, among a defined target consumer group, is usually the main goal of the product development process. Hence, comparison of consumer preference between prototype products and commercial products would hint on the commercial feasibility of the newly reformulated processed meat products.

Consumers in western countries are less familiar with seaweed as an ingredient in their diets. As such, developing our understanding of how cultural differences in perception, knowledge, and attitude influence food acceptance is imperative if we are to comprehend the behaviour of consumers towards products containing seaweed.

Finally, suitable future work expanding on the studies presented in this thesis could include those related to the beneficial effect of edible seaweeds and/or the final product on health; that is, the chemical analysis to identify and quantify bioactive compounds in the food matrix (seaweed), and the evaluation of the bioactivity, bioavailability, and functionality of bioactive compounds in the selected seaweed.

7.7. References

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Appendix

Appendix

Supplementary material

Chapter 3. A chemometric approach to characterize the aroma of selected brown and red edible seaweed / extracts.

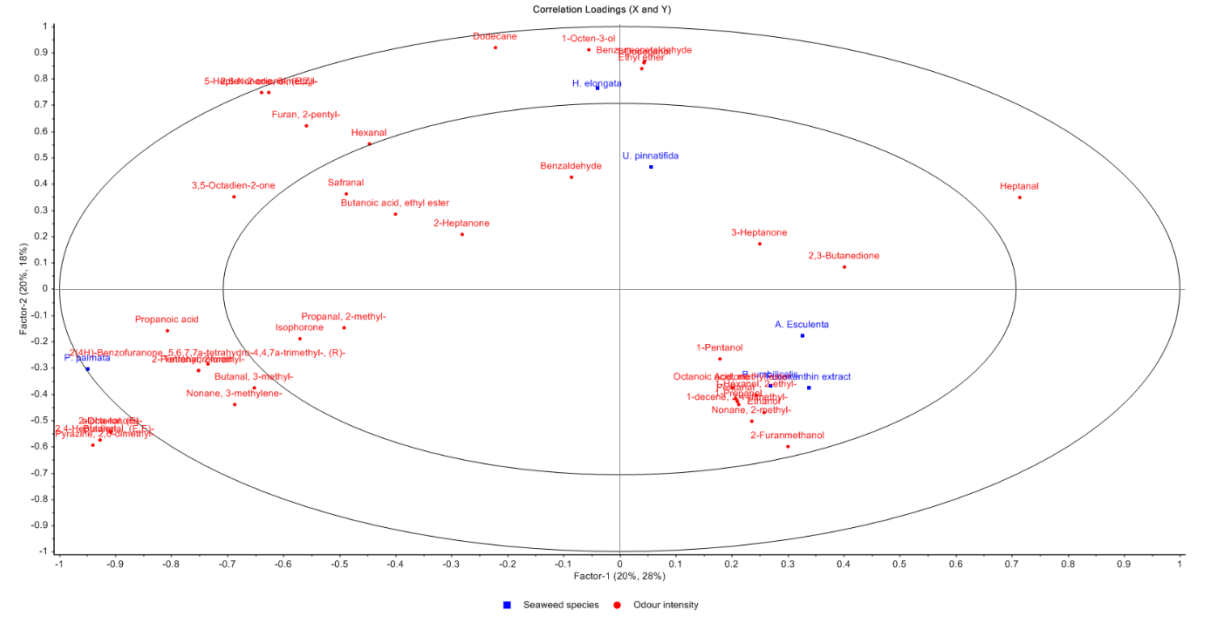


Figure S-3.1. Partial least squares regression (PLSR) correlation loadings plot for the volatile compounds identified by GC-O in different seaweed species (*H. elongata*, *U. pinnatifida*, *P. umbilicalis*, *P. palmata* and *A. esculenta*) and a seaweed extract (fucoxanthin extract from *L. japonica*). Shown are the X- (volatile compounds and their odour intensity values) and Y- (seaweed species and extract) variables for the first 2 PCs. • (red) = odour intensity values of each volatile compound, and ▪ (blue) = samples.

Table S-3.1. Volatile compounds identified in five species of edible seaweeds and a seaweed extract, after TD extraction.

Compound	Formula	CAS#	RI	IM	*H. elongata*	*U. pinnatifida*	*P. umbilicalis*	*P. palmata*	*A. esculenta*	Fucoxanthin extract	*p*
ACIDS											
Acetic acid	C2H4O2	64-19-7	687	MS, IHL, LRI	$2.75\times10^{6\,a}$	$7.39\times10^{6\,b}$	$8.86\times10^{4\,c}$	$2.64\times10^{6\,a}$	$1.68\times10^{6\,a}$	$2.05\times10^{6\,a}$	***
Propanoic acid	C3H6O2	79-09-4	780	MS, IHL, LRI	$2.51\times10^{5\,a}$	$1.98\times10^{6\,b}$	0.00×00	$9.76\times10^{5\,c}$	$1.18\times10^{5\,a}$	$1.02\times10^{5\,a}$	***
Propanoic acid, 2-methyl-	C4H8O2	79-31-2	835	MS, IHL, LRI	$2.19\times10^{5\,a}$	$2.07\times10^{6\,b}$	$3.82\times10^{4\,c}$	$5.43\times10^{5\,d}$	$4.47\times10^{5\,d}$	$1.34\times10^{5\,ac}$	***
Butanoic acid	C4H8O2	107-92-6	864	MS, IHL, LRI	$9.51\times10^{4\,a}$	$9.03\times10^{5\,b}$	0.00×00	$7.94\times10^{5\,b}$	$6.42\times10^{4\,a}$	$7.73\times10^{4\,a}$	***
Butanoic acid, 4-hydroxy-	C4H8O3	591-81-1	1029	MS	0.00×00	$2.48\times10^{5\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	***
Butanoic acid, 2-methyl-	C5H10O2	116-53-0	924	MS, IHL, LRI	0.00×00	$8.70\times10^{5\,a}$	0.00×00	$3.22\times10^{5\,b}$	$1.83\times10^{5\,c}$	$4.38\times10^{4\,d}$	***
Butanoic acid, 3-methyl-	C5H10O2	503-74-2	917	MS, IHL, LRI	$8.70\times10^{4\,a}$	$2.97\times10^{6\,b}$	$1.95\times10^{4\,a}$	$9.91\times10^{5\,c}$	$1.17\times10^{5\,a}$	$9.34\times10^{4\,a}$	***
Pentanoic acid	C5H10O2	109-52-4	958	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$5.94\times10^{4\,a}$	***
Propanoic acid, 2,2-dimethyl-	C5H10O2	75-98-9	876	MS, IHL	0.00×00	0.00×00	0.00×00	$2.77\times10^{5\,a}$	0.00×00	$3.80\times10^{4\,b}$	***
ALCOHOLS											
Ethanol	C2H6O	64-17-5	504	MS, IHL, LRI	0.00×00	$3.45\times10^{4\,a}$	0.00×00	0.00×00	$3.03\times10^{6\,b}$	$1.57\times10^{4\,a}$	***
2-propen-1-ol	C3H6O	107-18-6	606	MS	$2.77\times10^{4\,a}$	$1.92\times10^{4\,a}$	$1.67\times10^{4\,a}$	$1.79\times10^{4\,a}$	$9.10\times10^{5\,b}$	$3.51\times10^{3\,a}$	**
1-propanol	C3H8O	71-23-8	610	MS, IHL, LRI	$7.55\times10^{4\,a}$	$3.16\times10^{5\,b}$	$1.30\times10^{4\,c}$	$2.30\times10^{5\,d}$	$3.95\times10^{4\,c}$	0.00×00	***
Isopropyl Alcohol	C3H8O	67-63-0	540	MS	$4.83\times10^{5\,a}$	$8.21\times10^{5\,b}$	0.00×00	0.00×00	$2.28\times10^{5\,c}$	0.00×00	***
1-butanol	C4H10O	71-36-3	715	MS, IHL	$6.61\times10^{4\,a}$	$2.65\times10^{5\,b}$	$8.85\times10^{4\,c}$	$1.50\times10^{5\,d}$	$5.76\times10^{4\,a}$	$2.56\times10^{4\,e}$	***
2-butanol	C4H10O	78-92-2	647	MS, IHL, LRI	$1.07\times10^{4\,a}$	$4.45\times10^{4\,a}$	$1.21\times10^{4\,a}$	$5.55\times10^{4\,a}$	$1.40\times10^{4\,a}$	$2.41\times10^{4\,a}$	*
1-propanol, 2-methyl-	C4H10O	78-83-1	676	MS, IHL, LRI	0.00×00	$6.97\times10^{5\,a}$	$3.99\times10^{4\,b}$	$7.59\times10^{4\,bc}$	$9.17\times10^{4\,c}$	0.00×00	***
2-propanol, 1-methoxy-	C4H10O2	107-98-2	718	MS, IHL	$1.13\times10^{5\,abc}$	$3.05\times10^{5\,e}$	$1.13\times10^{5\,ac}$	$1.65\times10^{5\,ab}$	$4.95\times10^{4\,cd}$	0.00×00	***
2,3-butanediol	C4H10O2	513-85-9	779	MS, IHL, LRI	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$8.42\times10^{4\,a}$	ns
2-furanmethanol	C5H6O2	98-00-0	929	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$1.79\times10^{6\,a}$	***
1-penten-3-ol	C5H10O	616-25-1	732	MS	$6.88\times10^{6\,a}$	$3.33\times10^{6\,b}$	$6.76\times10^{5\,c}$	$1.04\times10^{7\,d}$	$7.36\times10^{6\,a}$	$1.00\times10^{5\,c}$	***
2-penten-1-ol, (Z)-	C5H10O	1576-95-0	822	MS	$1.75\times10^{6\,a}$	$3.64\times10^{5\,b}$	$8.51\times10^{4\,c}$	$1.47\times10^{6\,d}$	$1.52\times10^{6\,d}$	0.00×00	***
3-buten-2-ol, 2-methyl-	C5H10O	115-18-4	658	MS, LRI	$6.72\times10^{3\,a}$	$6.80\times10^{4\,b}$	0.00×00	$2.94\times10^{5\,c}$	0.00×00	0.00×00	***
1-pentanol	C5H12O	71-41-0	817	MS, IHL, LRI	$1.97\times10^{5\,a}$	$3.23\times10^{5\,b}$	$3.58\times10^{4\,c}$	$2.64\times10^{5\,d}$	$1.07\times10^{5\,e}$	$2.20\times10^{4\,c}$	***
1-butanol, 2-methyl-	C5H12O	137-32-6	789	MS, IHL, LRI	0.00×00	$3.45\times10^{5\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	***

1-butanol, 3-methyl-	C5H12O	123-51-3	796	MS, IHL, LRI	$9.62\times10^{4\,ab}$	$4.39\times10^{5\,c}$	$5.11\times10^{4\,a}$	$2.01\times10^{5\,b}$	$4.90\times10^{4\,a}$	0.00×00	***
Phenol	C6H6O	108-95-2	1097	MS, IHL, LRI	$5.35\times10^{4\,a}$	$6.60\times10^{4\,a}$	$6.85\times10^{4\,a}$	$7.39\times10^{4\,a}$	0.00×00	0.00×00	***
2-pentanol, 4-methyl-	C6H14O	108-11-2	805	MS	$1.14\times10^{7\,a}$	$1.09\times10^{7\,a}$	$1.14\times10^{7\,a}$	$1.13\times10^{7\,a}$	$9.52\times10^{6\,a}$	$7.54\times10^{6\,b}$	**
Ethanol, 2-butoxy-	C6H14O2	111-76-2	957	MS, LRI	$3.35\times10^{4\,a}$	0.00×00	$1.68\times10^{4\,a}$	0.00×00	$2.06\times10^{4\,a}$	$8.93\times10^{4\,a}$	ns
1-octen-3-ol	C8H16O	3391-86-4	1007	MS, IHL, LRI	$7.67\times10^{6\,a}$	$3.10\times10^{6\,ab}$	$1.92\times10^{5\,b}$	0.00×00	$7.64\times10^{6\,a}$	0.00×00	***
2-octen-1-ol, (E)-	C8H16O	18409-17-1	1124	MS	$1.99\times10^{5\,a}$	$1.74\times10^{5\,b}$	0.00×00	0.00×00	0.00×00	0.00×00	***
1-hexanol, 2-ethyl-	C8H18O	104-76-7	1079	MS, IHL, LRI	$1.13\times10^{5\,a}$	0.00×00	$3.17\times10^{5\,b}$	$1.36\times10^{5\,a}$	0.00×00	$2.40\times10^{5\,c}$	***
1-dodecanol	C12H26O	112-53-8	1473	MS, LRI	$6.22\times10^{3\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	ns
ALDEHYDES											
2-propenal	C3H4O	107-02-8	521	MS	$2.42\times10^{4\,a}$	$6.83\times10^{3\,a}$	$1.77\times10^{4\,a}$	$3.57\times10^{4\,a}$	$9.19\times10^{3\,a}$	0.00×00	ns
Propanal	C3H6O	123-38-6	526	MS, IHL, LRI	$3.14\times10^{5\,a}$	$3.34\times10^{5\,a}$	$1.86\times10^{5\,ac}$	$1.82\times10^{6\,d}$	$2.24\times10^{5\,ae}$	$6.69\times10^{4\,ce}$	***
Methacrolein	C4H6O	78-85-3	604	MS, IHL, LRI	$3.77\times10^{3\,a}$	$2.47\times10^{4\,b}$	$1.96\times10^{4\,b}$	$9.20\times10^{4\,c}$	0.00×00	$1.77\times10^{3\,a}$	***
Butanal	C4H8O	123-72-8	628	MS, LRI	$3.77\times10^{4\,a}$	$4.57\times10^{4\,a}$	$2.16\times10^{4\,a}$	$5.22\times10^{5\,b}$	$3.09\times10^{4\,a}$	0.00×00	***
Propanal, 2-methyl-	C4H8O	78-84-2	592	MS, IHL, LRI	$4.50\times10^{4\,a}$	$1.16\times10^{5\,b}$	$2.61\times10^{5\,c}$	$5.23\times10^{5\,d}$	$1.97\times10^{5\,e}$	$5.97\times10^{4\,a}$	***
Furfural	C5H4O2	98-01-1	901	MS, IHL, LRI	0.00×00	$1.87\times10^{4\,a}$	0.00×00	0.00×00	0.00×00	$7.00\times10^{5\,b}$	***
2-butenal, 2-methyl-	C5H8O	1115-11-3	672	MS	$1.67\times10^{4\,a}$	$1.39\times10^{4\,a}$	$2.98\times10^{4\,a}$	0.00×00	0.00×00	0.00×00	*
Pentanal	C5H10O	110-62-3	737	MS, IHL, LRI	$4.30\times10^{4\,a}$	$2.47\times10^{5\,b}$	$1.02\times10^{5\,a}$	$6.72\times10^{5\,c}$	$4.45\times10^{4\,a}$	$1.86\times10^{4\,a}$	***
Butanal, 2-methyl-	C5H10O	96-17-3	702	MS, IHL, LRI	0.00×00	0.00×00	$9.52\times10^{4\,a}$	$4.17\times10^{5\,b}$	0.00×00	$4.76\times10^{4\,a}$	***
Butanal, 3-methyl-	C5H10O	590-86-3	695	MS, IHL, LRI	$4.31\times10^{4\,a}$	0.00×00	$2.79\times10^{5\,b}$	$1.33\times10^{6\,c}$	$3.47\times10^{5\,d}$	$8.65\times10^{4\,e}$	***
2-hexenal, (E)-	C6H10O	6728-26-3	910	MS	$9.30\times10^{4\,a}$	$8.46\times10^{4\,b}$	$1.73\times10^{4\,c}$	$2.22\times10^{5\,d}$	$1.05\times10^{5\,e}$	0.00×00	***
2-pentenal, 2-methyl-	C6H10O	623-36-9	882	MS	0.00×00	0.00×00	0.00×00	$1.44\times10^{6\,a}$	0.00×00	0.00×00	***
Hexanal	C6H12O	66-25-1	842	MS, IHL, LRI	$1.05\times10^{6\,a}$	$9.78\times10^{5\,ab}$	$2.26\times10^{5\,c}$	$8.24\times10^{5\,b}$	$5.65\times10^{5\,d}$	$7.18\times10^{4\,e}$	***
Benzaldehyde	C7H6O	100-52-7	1034	MS	$2.19\times10^{5\,a}$	$2.41\times10^{5\,a}$	$2.18\times10^{5\,a}$	$5.13\times10^{5\,b}$	$5.22\times10^{5\,b}$	$6.55\times10^{4\,c}$	***
2,4-heptadienal, (E,E)-	C7H10O	4313-03-5	1079	MS	$2.64\times10^{5\,a}$	$4.57\times10^{4\,b}$	0.00×00	$7.35\times10^{5\,c}$	$1.39\times10^{5\,d}$	0.00×00	***
Heptanal	C7H14O	111-71-7	946	MS, IHL, LRI	$9.25\times10^{4\,a}$	$8.39\times10^{4\,a}$	$7.38\times10^{4\,a}$	$1.71\times10^{5\,b}$	0.00×00	$1.14\times10^{4\,c}$	***
Benzeneacetaldehyde	C8H8O	122-78-1	1123	MS, IHL, LRI	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$3.65\times10^{4\,a}$	***
Octanal	C8H16O	124-13-0	1050	MS, IHL, LRI	$1.86\times10^{5\,a}$	$2.08\times10^{5\,b}$	$4.00\times10^{4\,c}$	0.00×00	$8.64\times10^{4\,d}$	0.00×00	***
2,6-nonadienal, (E,Z)-	C9H14O	557-48-2	1225	MS	$4.04\times10^{4\,a}$	0.00×00	0.00×00	$8.67\times10^{4\,a}$	0.00×00	0.00×00	***
Nonanal	C9H18O	124-19-6	1151	MS, IHL, LRI	$8.67\times10^{4\,ab}$	$1.58\times10^{5\,b}$	$1.38\times10^{5\,ab}$	$1.26\times10^{5\,ab}$	$4.94\times10^{4\,a}$	$4.78\times10^{4\,a}$	**
BENZENES											
Benzene	C6H6	71-43-2	687	MS, IHL, LRI	0.00×00	$1.15\times10^{5\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	*

Toluene	C7H8	108-88-3	796	MS, IHL, LRI	$1.94\times10^{5\ ab}$	$1.16\times10^{5\ ab}$	$1.60\times10^{5\ ab}$	$1.78\times10^{5\ ab}$	$3.72\times10^{5\ a}$	$7.85\times10^{4\ b}$	*
Styrene	C8H8	100-42-5	932	MS, IHL	$3.90\times10^{4\ a}$	$3.12\times10^{4\ a}$	$2.68\times10^{4\ a}$	$6.89\times10^{4\ b}$	0.00×00	0.00×00	***
Ethylbenzene	C8H10	100-41-4	877	MS, IHL, LRI	$1.33\times10^{5\ a}$	$6.57\times10^{5\ b}$	$8.77\times10^{4\ a}$	0.00×00	$1.94\times10^{5\ a}$	0.00×00	***
p-xylene	C8H10	106-42-3	902	MS, IHL	$5.91\times10^{4\ a}$	$4.62\times10^{4\ ab}$	$4.23\times10^{4\ ad}$	$5.55\times10^{4\ a}$	$9.21\times10^{4\ c}$	$2.94\times10^{4\ bd}$	***
1,3-di-tert-butylbenzene	C14H22	1014-60-4	1292	MS, IHL	0.00×00	0.00×00	$6.25\times10^{3\ a}$	$1.79\times10^{3\ a}$	$4.60\times10^{2\ a}$	$5.55\times10^{6\ b}$	***
ESTERS and ETHERS											
Acetic acid, methyl ester	C3H6O2	79-20-9	555	MS, LRI	0.00×00	$1.81\times10^{5\ a}$	0.00×00	$8.23\times10^{5\ b}$	0.00×00	$1.98\times10^{4\ c}$	***
Ethyl acetate	C4H8O2	141-78-6	642	MS, IHL, LRI	$3.20\times10^{5\ a}$	$4.04\times10^{5\ b}$	$4.21\times10^{5\ b}$	$1.40\times10^{6\ c}$	$4.37\times10^{5\ b}$	$2.55\times10^{5\ a}$	***
Ethyl ether	C4H10O	60-29-7	514	MS, IHL	0.00×00	0.00×00	0.00×00	0.00×00	$5.07\times10^{6\ a}$	0.00×00	***
Butanoic acid, methyl ester	C5H10O2	623-42-7	751	MS, IHL, LRI	0.00×00	0.00×00	0.00×00	$5.00\times10^{4\ a}$	$1.24\times10^{4\ b}$	0.00×00	***
n-propyl acetate	C5H10O2	109-60-4	744	MS, IHL, LRI	$1.46\times10^{4\ a}$	$8.05\times10^{4\ b}$	0.00×00	0.00×00	$1.96\times10^{4\ a}$	0.00×00	***
Butanedioic acid, dimethyl ester	C6H10O4	106-65-0	1086	MS	$4.58\times10^{3\ a}$	$1.44\times10^{4\ a}$	$4.30\times10^{3\ a}$	0.00×00	$4.07\times10^{3\ a}$	$9.58\times10^{3\ a}$	*
1-methoxy-2-propyl acetate	C6H12O3	108-65-6	903	MS	$8.61\times10^{4\ a}$	0.00×00	$6.05\times10^{4\ b}$	0.00×00	0.00×00	$4.98\times10^{4\ b}$	***
Pentanedioic acid, dimethyl ester	C7H12O4	1119-40-0	1193	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$1.11\times10^{4\ a}$	***
tert-butyl glycidyl ether	C7H14O2	7665-72-7	1046	MS	$6.94\times10^{4\ ac}$	$2.69\times10^{5\ b}$	$1.40\times10^{5\ c}$	$9.36\times10^{4\ ac}$	$5.75\times10^{4\ a}$	0.00×00	***
Dimethyl phthalate	C10H10O4	131-11-3	1565	MS	0.00×00	0.00×00	$1.92\times10^{4\ a}$	$1.02\times10^{5\ b}$	0.00×00	0.00×00	***
Diethyl phthalate	C12H14O4	84-66-2	1702	MS	$1.61\times10^{4\ a}$	$2.44\times10^{4\ a}$	$1.55\times10^{4\ a}$	$1.24\times10^{4\ a}$	$6.19\times10^{3\ a}$	$4.51\times10^{3\ a}$	*
FURANS											
Furan	C4H4O	110-00-9	516	MS	0.00×00	0.00×00	$3.26\times10^{3\ a}$	$2.22\times10^{4\ a}$	0.00×00	$6.96\times10^{4\ b}$	***
Tetrahydrofuran	C4H8O	109-99-9	655	MS, LRI	0.00×00	0.00×00	0.00×00	0.00×00	$5.30\times10^{5\ a}$	0.00×00	***
Furan, 2-methyl-	C5H6O	534-22-5	622	MS, LRI	$6.45\times10^{3\ a}$	$4.46\times10^{3\ a}$	0.00×00	$3.66\times10^{4\ bc}$	$1.62\times10^{4\ ab}$	$5.85\times10^{4\ c}$	***
Furan, 2-acetyl-	C6H6O2	1192-62-7	985	MS	0.00×00	$6.30\times10^{3\ a}$	0.00×00	$2.05\times10^{3\ a}$	$2.92\times10^{3\ a}$	$1.46\times10^{5\ b}$	***
Furfural, 5-methyl-	C6H6O2	620-02-0	1040	MS, IHL	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$1.02\times10^{5\ a}$	***
Furan, 2-ethyl-	C6H8O	3208-16-0	721	MS, IHL, LRI	$7.26\times10^{4\ a}$	$9.80\times10^{4\ a}$	0.00×00	$5.09\times10^{5\ b}$	$3.23\times10^{5\ c}$	$1.85\times10^{4\ d}$	***
Furan, 2-pentyl-	C9H14O	3777-69-3	1016	MS, IHL, LRI	$2.13\times10^{5\ a}$	$1.95\times10^{5\ a}$	0.00×00	$3.84\times10^{4\ b}$	$6.06\times10^{5\ c}$	0.00×00	***
KETONES											
Acetone	C3H6O	67-64-1	531	MS, IHL, LRI	$1.87\times10^{6\ a}$	$5.06\times10^{5\ a}$	$1.81\times10^{6\ a}$	$1.61\times10^{6\ a}$	$1.26\times10^{6\ a}$	$8.31\times10^{5\ a}$	*
2-propanone, 1-hydroxy-	C3H6O2	116-09-6	734	MS, IHL	$3.15\times10^{5\ a}$	0.00×00	0.00×00	0.00×00	0.00×00	$1.54\times10^{6\ b}$	***
2,3-butanedione	C4H6O2	431-03-8	630	MS, IHL, LRI	$7.26\times10^{4\ a}$	$8.66\times10^{4\ a}$	$4.29\times10^{5\ a}$	$3.87\times10^{5\ a}$	$2.07\times10^{5\ a}$	$2.45\times10^{4\ a}$	*

2-butanone	C4H8O	78-93-3	638	MS, IHL, LRI	$3.27\times10^{5\ a}$	$4.19\times10^{5\ b}$	$3.68\times10^{5\ ab}$	$1.23\times10^{6\ c}$	$3.92\times10^{5\ ab}$	$2.23\times10^{5\ d}$	***
Acetoin	C4H8O2	513-86-0	779	MS, IHL, LRI	$1.38\times10^{5\ a}$	$3.05\times10^{6\ b}$	$6.17\times10^{5\ cd}$	$2.98\times10^{6\ b}$	$1.08\times10^{6\ d}$	$1.93\times10^{5\ ac}$	***
2-cyclopenten-1-one	C5H6O	930-30-3	905	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$9.98\times10^{4\ a}$	***
1,2-cyclopentanedione	C5H6O2	3008-40-0	944	MS	$4.69\times10^{4\ a}$	$2.17\times10^{5\ a}$	$8.51\times10^{3\ a}$	0.00×00	$9.85\times10^{4\ a}$	$8.60\times10^{3\ a}$	ns
1-penten-3-one	C5H8O	1629-58-9	720	MS	$1.99\times10^{4\ a}$	$1.57\times10^{4\ a}$	$4.03\times10^{4\ a}$	$4.40\times10^{5\ b}$	0.00×00	0.00×00	***
3-penten-2-one	C5H8O	625-33-2	791	MS	$2.42\times10^{4\ a}$	$5.17\times10^{4\ b}$	$9.49\times10^{3\ a}$	$3.37\times10^{5\ c}$	$6.96\times10^{4\ d}$	$1.13\times10^{4\ a}$	***
Cyclopentanone	C5H8O	120-92-3	851	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$2.25\times10^{4\ a}$	***
3-pentanone	C5H10O	96-22-0	738	MS, LRI	0.00×00	0.00×00	0.00×00	0.00×00	$1.04\times10^{5\ a}$	0.00×00	ns
Methyl isobutyl ketone	C6H12O	108-10-1	783	MS, IHL	$2.48\times10^{4\ a}$	0.00×00	$7.03\times10^{4\ b}$	0.00×00	$5.64\times10^{4\ ab}$	$6.94\times10^{3\ a}$	***
1H-pyrrole-2,5-dione, 3-ethyl-4-methyl-	C7H9NO2	20189-42-8	1349	MS	0.00×00	$7.67\times10^{4\ a}$	0.00×00	$2.69\times10^{4\ b}$	$1.80\times10^{4\ b}$	0.00×00	***
2-heptanone	C7H14O	110-43-0	937	MS, IHL, LRI	$3.13\times10^{4\ a}$	$9.23\times10^{4\ b}$	0.00×00	0.00×00	$1.22\times10^{5\ c}$	0.00×00	***
Acetophenone	C8H8O	98-86-2	1070	MS, IHL	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$9.98\times10^{3\ a}$	***
3,5-octadien-2-one	C8H12O	38284-27-4	1136	MS	$3.93\times10^{5\ a}$	$1.17\times10^{5\ b}$	$3.09\times10^{4\ c}$	$1.42\times10^{6\ d}$	$2.83\times10^{5\ e}$	0.00×00	***
3,5-octadien-2-one, (E,E)-	C8H12O	30086-02-3	1165	MS	$5.07\times10^{4\ a}$	$4.48\times10^{4\ a}$	$1.23\times10^{4\ b}$	$3.21\times10^{5\ c}$	$9.37\times10^{4\ d}$	0.00×00	***
5-hepten-2-one, 6-methyl-	C8H14O	110-93-0	1036	MS, IHL, LRI	$1.37\times10^{5\ ab}$	$2.88\times10^{5\ c}$	$1.32\times10^{5\ ab}$	$1.47\times10^{5\ a}$	$1.17\times10^{5\ b}$	0.00×00	***
2-octanone	C8H16O	111-13-7	1040	MS	$2.66\times10^{4\ a}$	$3.81\times10^{4\ ab}$	$8.69\times10^{3\ c}$	0.00×00	$4.20\times10^{4\ b}$	0.00×00	***
Isophorone	C9H14O	78-59-1	1123	MS	0.00×00	0.00×00	0.00×00	$3.66\times10^{5\ a}$	0.00×00	0.00×00	*
LACTONES											
2(5H)-furanone	C4H4O2	497-23-4	1033	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$1.18\times10^{5\ a}$	***
2(5H)-furanone, 5-methyl-	C5H6O2	591-11-7	1054	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$5.16\times10^{4\ a}$	***
2(3H)-furanone, 5-ethyldihydro-	C6H10O2	695-06-7	1173	MS	0.00×00	$1.84\times10^{5\ a}$	0.00×00	$8.40\times10^{4\ b}$	$1.01\times10^{5\ c}$	$3.06\times10^{4\ d}$	***
PYRAZINES and PYRIDINES											
Pyrazine	C4H4N2	290-37-9	774	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$1.13\times10^{5\ a}$	***
Pyridine	C5H5N	110-86-1	790	MS, IHL, LRI	0.00×00	$1.96\times10^{4\ a}$	0.00×00	$1.42\times10^{4\ a}$	0.00×00	$2.01\times10^{4\ a}$	**
Pyrazine, methyl-	C5H6N2	109-08-0	866	MS, LRI	0.00×00	0.00×00	0.00×00	$4.08\times10^{4\ a}$	0.00×00	$1.23\times10^{5\ b}$	***
Pyridine, 3-methyl-	C6H7N	108-99-6	915	MS	0.00×00	0.00×00	0.00×00	$1.19\times10^{5\ a}$	0.00×00	0.00×00	***
Pyrazine, 2,5-dimethyl-	C6H8N2	123-32-0	954	MS, IHL	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$2.75\times10^{4\ a}$	***
Pyrazine, 2,6-dimethyl-	C6H8N2	108-50-9	954	MS, LRI	0.00×00	0.00×00	0.00×00	$3.30\times10^{5\ a}$	0.00×00	0.00×00	***
Pyrazine, tetramethyl-	C8H12N2	1124-11-4	1127	MS, IHL, LRI	0.00×00	$1.23\times10^{5\ a}$	0.00×00	0.00×00	0.00×00	0.00×00	***

SULFUR COMPOUNDS											
Sulfur dioxide	O2S	7446-09-5	319	MS	$9.17\times10^{5\ abc}$	$9.40\times10^{5\ ab}$	$7.36\times10^{5\ abc}$	$1.09\times10^{6\ a}$	$3.56\times10^{5\ bc}$	$2.83\times10^{5\ c}$	**
Carbon disulfide	CS2	75-15-0	546	MS, IHL, LRI	$2.59\times10^{4\ a}$	0.00×00	0.00×00	$3.44\times10^{4\ b}$	$2.28\times10^{4\ a}$	0.00×00	***
Dimethyl sulfide	C2H6S	75-18-3	537	MS, IHL, LRI	$4.17\times10^{3\ a}$	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	**
Dimethyl disulfide	C2H6S2	624-92-0	781	MS, LRI	$1.11\times10^{4\ a}$	0.00×00	$6.14\times10^{4\ b}$	$8.37\times10^{4\ b}$	$5.76\times10^{4\ b}$	$3.50\times10^{3\ a}$	***
TERPENES											
Safranal	C10H14O	116-26-7	1282	MS	$4.64\times10^{3\ a}$	$4.67\times10^{3\ a}$	0.00×00	$1.75\times10^{4\ b}$	$4.42\times10^{3\ a}$	0.00×00	***
D-limonene	C10H16	5989-27-5	1053	MS, IHL, LRI	$3.82\times10^{5\ a}$	$3.35\times10^{4\ b}$	$4.56\times10^{4\ b}$	$1.82\times10^{5\ c}$	$1.74\times10^{5\ c}$	$5.08\times10^{3\ b}$	***
Dihydroactinidiolide	C11H16O2	17092-92-1	1731	MS	$1.09\times10^{4\ a}$	$5.91\times10^{4\ b}$	$5.21\times10^{3\ a}$	$3.22\times10^{4\ c}$	$4.80\times10^{3\ a}$	0.00×00	***
alpha-Ionone	C13H20O	127-41-3	1523	MS	0.00×00	0.00×00	0.00×00	$4.55\times10^{5\ a}$	0.00×00	0.00×00	***
(E)-beta-ionone	C13H20O	79-77-6	1584	MS	$4.92\times10^{4\ a}$	$1.58\times10^{4\ b}$	$1.07\times10^{4\ b}$	$1.99\times10^{5\ c}$	$3.77\times10^{4\ a}$	0.00×00	***
OTHERS											
Trichloromethane	CHCl3	67-66-3	655	MS, IHL	0.00×00	0.00×00	$3.54\times10^{4\ a}$	$3.98\times10^{4\ a}$	$1.64\times10^{4\ b}$	0.00×00	***
2,4-di-tert-butylphenol	C14H22O	96-76-4	1603	MS, IHL	$4.16\times10^{3\ a}$	0.00×00	$1.44\times10^{4\ a}$	$1.95\times10^{4\ a}$	0.00×00	$5.76\times10^{4\ b}$	***

Levels of volatile compounds are expressed as abundances (mean values from 3 determinations from each seaweed). RT and RI, retention time and retention index on a DB-624 UI column; IM, identification method; MS, spectra comparison using NIST mass spectral database; IHL, in-house library created using authentic compounds with target and qualifier ions and linear RI for each compound; LRI, RI agree with literature values. ns, not significant; *, P <0.05; **, P <0.01; ***, P <0.001, significance of seaweed species according to ANOVA. $^{a\text{-}e}$, means in the same row bearing different superscript letter denote statistical significant differences (P <0.05).

Table S-3.2. Volatile compounds identified in five species of edible seaweeds and a seaweed extract, after HS-SPME.

Name	Formula	CAS#	RI	IM	*H. elongata*	*U. pinnatifida*	*P. umbilicalis*	*P. palmata*	*A. esculenta*	Fucoxanthin extract	*p*
ACIDS											
Acetic acid	C2H4O2	64-19-7	689	MS, IHL, LRI	$3.90\times10^{6\ a}$	$1.19\times10^{7\ b}$	0.00×00	$7.53\times10^{6\ c}$	$6.20\times10^{6\ d}$	$7.19\times10^{6\ cd}$	***
Propanoic acid	C3H6O2	79-09-4	780	MS, IHL, LRI	$1.32\times10^{5\ a}$	$1.15\times10^{6\ b}$	0.00×00	$1.22\times10^{6\ b}$	0.00×00	$3.42\times10^{5\ c}$	***
Propanoic acid, 2-methyl-	C4H8O2	79-31-2	835	MS, IHL, LRI	$2.70\times10^{5\ a}$	$2.57\times10^{6\ b}$	0.00×00	$9.84\times10^{5\ c}$	$1.37\times10^{6\ d}$	$2.79\times10^{5\ a}$	***
Butanoic acid	C4H8O2	107-92-6	864	MS, IHL, LRI	$7.98\times10^{4\ a}$	$9.52\times10^{5\ a}$	0.00×00	$2.40\times10^{6\ b}$	$2.33\times10^{5\ a}$	$2.23\times10^{5\ a}$	***
Butanoic acid, 2-methyl-	C5H10O2	116-53-0	925	MS, IHL, LRI	$1.82\times10^{4\ a}$	$8.56\times10^{5\ b}$	0.00×00	$1.02\times10^{6\ c}$	$6.96\times10^{5\ d}$	$8.45\times10^{4\ a}$	***
Butanoic acid, 3-methyl-	C5H10O2	503-74-2	917	MS, IHL, LRI	$1.47\times10^{5\ a}$	$2.81\times10^{6\ b}$	0.00×00	$2.46\times10^{6\ c}$	$1.23\times10^{6\ d}$	$1.98\times10^{5\ a}$	***
Pentanoic acid	C5H10O2	109-52-4	958	MS	0.00×00	$3.99\times10^{5\ a}$	0.00×00	0.00×00	0.00×00	$5.21\times10^{5\ b}$	***
Propanoic acid, 2,2-dimethyl-	C5H10O2	75-98-9	876	MS, IHL	0.00×00	0.00×00	$4.31\times10^{4\ a}$	0.00×00	0.00×00	$1.79\times10^{5\ b}$	**
Hexanoic acid	C6H12O2	142-62-1	1054	MS, IHL, LRI	0.00×00	$2.24\times10^{6\ a}$	0.00×00	0.00×00	0.00×00	$1.64\times10^{6\ b}$	***
ALCOHOLS											
Ethanol	C2H6O	64-17-5	511	MS, IHL, LRI	0.00×00	0.00×00	0.00×00	$2.65\times10^{5\ a}$	0.00×00	$7.87\times10^{4\ b}$	***
1-propanol	C3H8O	71-23-8	612	MS, IHL, LRI	$1.64\times10^{4\ a}$	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	***
Isopropyl alcohol	C3H8O	67-63-0	543	MS	$1.38\times10^{4\ a}$	$1.33\times10^{4\ a}$	0.00×00	0.00×00	0.00×00	$4.87\times10^{3\ a}$	**
1-butanol	C4H10O	71-36-3	702	MS, IHL	$2.00\times10^{5\ a}$	$3.85\times10^{4\ a}$	0.00×00	0.00×00	$1.63\times10^{6\ b}$	$8.61\times10^{3\ a}$	***
1-propanol, 2-methyl	C4H10O	78-83-1	679	MS, IHL, LRI	0.00×00	$3.45\times10^{4\ a}$	$6.91\times10^{3\ b}$	$4.84\times10^{3\ b}$	0.00×00	0.00×00	***
2-propanol, 1-methoxy-	C4H10O2	107-98-2	720	MS, IHL	$6.11\times10^{3\ a}$	$6.80\times10^{4\ ab}$	$1.03\times10^{5\ bc}$	$3.81\times10^{4\ ac}$	$1.71\times10^{4\ a}$	0.00×00	**
2-furanmethanol	C5H6O2	98-00-0	930	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$1.10\times10^{6\ a}$	***
1-penten-3-ol	C5H10O	616-25-1	731	MS	$8.98\times10^{6\ a}$	$9.81\times10^{5\ b}$	$8.11\times10^{5\ b}$	0.00×00	$5.61\times10^{6\ ab}$	0.00×00	**

2-penten-1-ol, (Z)-	C5H10O	1576-95-0	824	MS	$9.67\times10^{5\ a}$	$3.06\times10^{4\ b}$	0.00×00	$9.81\times10^{5\ a}$	$1.44\times10^{6\ a}$	0.00×00	***
3-buten-2-ol, 2-methyl-	C5H10O	115-18-4	661	MS, LRI	0.00×00	0.00×00	0.00×00	$1.34\times10^{5\ a}$	0.00×00	0.00×00	***
1-pentanol	C5H12O	71-41-0	796	MS, IHL, LRI	$1.23\times10^{5\ a}$	$4.62\times10^{5\ ab}$	$9.33\times10^{4\ a}$	$6.44\times10^{5\ b}$	$5.57\times10^{5\ b}$	0.00×00	***
Phenol	C6H6O	108-95-2	1098	MS, IHL, LRI	0.00×00	$2.73\times10^{4\ a}$	0.00×00	0.00×00	0.00×00	$3.02\times10^{4\ a}$	*
1-octen-3-ol	C8H16O	3391-86-4	1023	MS, IHL, LRI	$1.34\times10^{6\ a}$	$8.59\times10^{6\ b}$	$1.01\times10^{6\ a}$	$1.64\times10^{6\ a}$	$1.82\times10^{6\ a}$	$1.94\times10^{6\ a}$	***
1-hexanol, 2-ethyl-	C8H18O	104-76-7	1071	MS, IHL, LRI	$1.23\times10^{6\ ab}$	$1.52\times10^{6\ abc}$	$3.00\times10^{6\ d}$	$2.40\times10^{6\ bcd}$	$2.56\times10^{6\ cd}$	$5.34\times10^{5\ a}$	***
1-octanol, 2-butyl-	C12H26O	3913-02-8	1304	MS	$7.84\times10^{4\ ab}$	$3.35\times10^{4\ a}$	$1.77\times10^{5\ c}$	0.00×00	0.00×00	$1.09\times10^{5\ b}$	***
1-dodecanol	C12H26O	112-53-8	1504	MS, LRI	$5.79\times10^{4\ a}$	0.00×00	0.00×00	0.00×00	$1.12\times10^{5\ a}$	0.00×00	ns
ALDEHYDES											
Acetaldehyde	C2H4O	75-07-0	378	MS, IHL, LRI	0.00×00	$2.74\times10^{5\ a}$	$2.14\times10^{6\ b}$	$1.18\times10^{6\ c}$	$1.57\times10^{5\ a}$	$1.35\times10^{5\ a}$	***
Propanal	C3H6O	123-38-6	530	MS, IHL, LRI	$2.97\times10^{5\ a}$	$1.55\times10^{5\ b}$	$2.97\times10^{5\ a}$	$7.79\times10^{6\ c}$	$2.46\times10^{5\ ab}$	0.00×00	***
Methacrolein	C4H6O	78-85-3	609	MS, IHL, LRI	0.00×00	0.00×00	0.00×00	$3.26\times10^{5\ a}$	0.00×00	0.00×00	***
Butanal	C4H8O	123-72-8	631	MS, LRI	$4.11\times10^{4\ a}$	0.00×00	$4.65\times10^{4\ a}$	$6.66\times10^{5\ b}$	0.00×00	0.00×00	***
Propanal, 2-methyl-	C4H8O	78-84-2	597	MS, IHL, LRI	$1.21\times10^{4\ a}$	0.00×00	$2.85\times10^{5\ b}$	$2.35\times10^{5\ b}$	$5.32\times10^{4\ a}$	0.00×00	***
Furfural	C5H4O2	98-01-1	892	MS, IHL, LRI	0.00×00	0.00×00	0.00×00	$1.47\times10^{4\ a}$	$5.84\times10^{3\ a}$	$2.58\times10^{5\ b}$	***
2-pentenal, (E)-	C5H8O	1576-87-0	808	MS	$6.92\times10^{4\ a}$	$6.98\times10^{4\ a}$	$3.24\times10^{4\ a}$	$1.22\times10^{6\ b}$	$2.71\times10^{5\ c}$	0.00×00	***
Pentanal	C5H10O	110-62-3	736	MS, IHL, LRI	$1.37\times10^{5\ abc}$	$2.97\times10^{5\ abc}$	$3.53\times10^{5\ ab}$	$2.19\times10^{6\ d}$	$2.83\times10^{5\ bc}$	$5.09\times10^{4\ c}$	***
Butanal, 2-methyl-	C5H10O	96-17-3	704	MS, IHL, LRI	0.00×00	0.00×00	$4.34\times10^{5\ a}$	$7.83\times10^{5\ b}$	0.00×00	0.00×00	***
Butanal, 3-methyl-	C5H10O	590-86-3	695	MS, IHL, LRI	$7.00\times10^{4\ a}$	0.00×00	$9.49\times10^{5\ b}$	$1.92\times10^{6\ c}$	$2.70\times10^{5\ a}$	$4.62\times10^{4\ a}$	***
2-pentenal, 2-methyl-	C6H10O	623-36-9	884	MS	0.00×00	0.00×00	0.00×00	$4.13\times10^{6\ a}$	0.00×00	0.00×00	***
2-hexenal, (E)-	C6H10O	6728-26-3	907	MS	$1.63\times10^{5\ a}$	$1.54\times10^{5\ a}$	$4.46\times10^{4\ b}$	$9.32\times10^{5\ c}$	$3.43\times10^{5\ d}$	0.00×00	***

Hexanal	C6H12O	66-25-1	842	MS, IHL, LRI	$3.05\times10^{6\ ab}$	$1.66\times10^{6\ c}$	$7.64\times10^{5\ d}$	$3.72\times10^{6\ a}$	$2.41\times10^{6\ b}$	$2.92\times10^{5\ d}$	***
Benzaldehyde	C7H6O	100-52-7	1034	MS, IHL, LRI	$7.01\times10^{4\ a}$	$7.10\times10^{4\ a}$	$7.51\times10^{4\ a}$	$4.83\times10^{5\ b}$	$6.72\times10^{5\ c}$	$7.62\times10^{3\ d}$	***
2,4-heptadienal, (E,E)-	C7H10O	4313-03-5	1082	MS	$2.17\times10^{5\ a}$	$7.25\times10^{4\ b}$	0.00×00	$1.45\times10^{6\ c}$	$2.70\times10^{5\ a}$	0.00×00	***
Heptanal	C7H14O	111-71-7	946	MS, IHL, LRI	$1.80\times10^{5\ a}$	0.00×00	$2.37\times10^{5\ a}$	$8.49\times10^{5\ b}$	0.00×00	$6.39\times10^{4\ c}$	***
Octanal	C8H16O	124-13-0	1073	MS, IHL, LRI	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$5.25\times10^{5\ a}$	***
Benzaldehyde, 2,4-dimethyl-	C9H10O	15764-16-6	1316	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$5.35\times10^{4\ a}$	***
2,6-nonadienal, (E,Z)-	C9H14O	557-48-2	1225	MS	$1.78\times10^{5\ a}$	0.00×00	0.00×00	$6.14\times10^{5\ b}$	0.00×00	0.00×00	***
Nonanal	C9H18O	124-19-6	1136	MS, IHL, LRI	$4.41\times10^{5\ a}$	$1.32\times10^{5\ b}$	$2.85\times10^{5\ c}$	$1.06\times10^{6\ d}$	$1.12\times10^{5\ b}$	$9.76\times10^{4\ b}$	***
BENZENES											
Benzene	C6H6	71-43-2	689	MS, IHL, LRI	0.00×00	$1.98\times10^{4\ a}$	0.00×00	$7.16\times10^{4\ b}$	$3.14\times10^{4\ a}$	0.00×00	***
Toluene	C7H8	108-88-3	796	MS, IHL, LRI	$9.23\times10^{4\ abc}$	$1.57\times10^{5\ ab}$	$8.60\times10^{4\ abc}$	$1.73\times10^{5\ b}$	$7.07\times10^{5\ d}$	$2.19\times10^{4\ c}$	***
Styrene	C8H8	100-42-5	932	MS, IHL	$2.17\times10^{4\ a}$	$1.17\times10^{4\ ab}$	$1.98\times10^{4\ ac}$	$1.73\times10^{5\ d}$	0.00×00	$2.96\times10^{3\ bc}$	***
Ethylbenzene	C8H10	100-41-4	894	MS, IHL, LRI	$1.66\times10^{4\ a}$	0.00×00	$3.56\times10^{3\ a}$	$3.33\times10^{4\ a}$	$7.43\times10^{4\ b}$	$1.92\times10^{4\ a}$	***
o-xylene	C8H10	95-47-6	931	MS, LRI	$1.71\times10^{4\ a}$	$3.97\times10^{3\ a}$	$1.98\times10^{4\ a}$	$1.77\times10^{4\ a}$	$2.00\times10^{5\ b}$	$3.05\times10^{4\ a}$	***
p-xylene	C8H10	106-42-3	901	MS, IHL	$3.84\times10^{4\ a}$	$1.37\times10^{4\ a}$	0.00×00	$7.86\times10^{4\ a}$	$7.15\times10^{4\ a}$	0.00×00	ns
Cumene	C9H12	98-82-8	997	MS	$1.29\times10^{4\ a}$	$6.68\times10^{3\ a}$	$7.47\times10^{3\ a}$	$2.06\times10^{4\ ab}$	$1.22\times10^{5\ c}$	$3.20\times10^{4\ b}$	***
Mesitylene	C9H12	108-67-8	1024	MS	0.00×00	$4.45\times10^{3\ a}$	$3.69\times10^{3\ a}$	0.00×00	$9.71\times10^{4\ b}$	$5.99\times10^{4\ ab}$	**
1,3-di-tert-butylbenzene	C14H22	1014-60-4	1293	MS, IHL	3.46×10^{3a}	0.00×00	$7.99\times10^{3\ a}$	$1.51\times10^{4\ a}$	0.00×00	$1.82\times10^{7\ b}$	***
ESTERS and ETHERS											
Acetic acid, methyl ester	C3H6O2	79-20-9	560	MS, LRI	0.00×00	0.00×00	0.00×00	$1.44\times10^{6\ a}$	0.00×00	0.00×00	***
Methyl hydroxyacetate	C3H6O3	96-35-5	762	MS	0.00×00	0.00×00	0.00×00	$2.24\times10^{4\ a}$	0.00×00	0.00×00	***

Ethyl acetate	C4H8O2	141-78-6	642	MS, IHL, LRI	0.00×00	$3.88\times10^{2\,a}$	$6.80\times10^{3\,b}$	$6.39\times10^{3\,b}$	$3.16\times10^{3\,c}$	0.00×00	***
Ethyl ether	C4H10O	60-29-7	518	MS, IHL	$4.38\times10^{5\,a}$	$1.17\times10^{5\,a}$	$1.57\times10^{5\,a}$	$4.77\times10^{5\,a}$	$8.44\times10^{6\,b}$	0.00×00	***
Butanoic acid, methyl ester	C5H10O2	623-42-7	752	MS, IHL, LRI	0.00×00	0.00×00	$1.57\times10^{4\,a}$	$9.81\times10^{4\,b}$	$6.46\times10^{4\,b}$	0.00×00	***
Butanoic acid, ethyl ester	C6H12O2	105-54-4	829	MS, IHL, LRI	0.00×00	0.00×00	0.00×00	0.00×00	$7.76\times10^{4\,a}$	0.00×00	***
tert-butyl glycidyl ether	C7H14O2	7665-72-7	1044	MS	$1.46\times10^{5\,a}$	$3.62\times10^{5\,b}$	$6.36\times10^{5\,c}$	$3.82\times10^{5\,b}$	$3.04\times10^{5\,ab}$	$3.78\times10^{3\,a}$	***
Dimethyl phthalate	C10H10O4	131-11-3	1568	MS	0.00×00	0.00×00	$1.42\times10^{4\,a}$	$1.32\times10^{5\,b}$	0.00×00	0.00×00	***
Propanoic acid, 2-methyl-, 3-hydroxy-2,2,4-trimethylpentyl ester	C12H24O3	77-68-9	1467	MS	0.00×00	$7.65\times10^{4\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	***
FURANS											
Furan	C4H4O	110-00-9	519	MS	$1.84\times10^{4\,a}$	$1.01\times10^{3\,a}$	$6.53\times10^{3\,a}$	$1.95\times10^{4\,a}$	$1.60\times10^{4\,a}$	$1.34\times10^{4\,a}$	ns
Tetrahydrofuran	C4H8O	109-99-9	657	MS, LRI	$4.91\times10^{4\,a}$	0.00×00	$1.73\times10^{5\,b}$	$1.42\times10^{5\,b}$	$2.92\times10^{5\,c}$	0.00×00	***
Furan, 2-methyl-	C5H6O	534-22-5	625	MS, LRI	$2.12\times10^{4\,a}$	$1.02\times10^{4\,a}$	$1.08\times10^{4\,a}$	$1.06\times10^{5\,b}$	$5.47\times10^{4\,c}$	$1.34\times10^{4\,a}$	***
Furfural, 5-methyl-	C6H6O2	620-02-0	1042	MS, IHL	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$3.15\times10^{4\,a}$	***
Furan, 2-ethyl-	C6H8O	3208-16-0	722	MS, IHL, LRI	$2.67\times10^{5\,a}$	$2.08\times10^{5\,a}$	$4.52\times10^{4\,a}$	$1.56\times10^{6\,b}$	$8.28\times10^{5\,c}$	0.00×00	***
Furan, 2-pentyl-	C9H14O	3777-69-3	1016	MS, IHL, LRI	$4.94\times10^{5\,a}$	$3.31\times10^{5\,a}$	0.00×00	$1.79\times10^{5\,a}$	$2.74\times10^{6\,b}$	0.00×00	***
KETONES											
Acetone	C3H6O	67-64-1	535	MS, IHL, LRI	$2.16\times10^{6\,a}$	$1.90\times10^{5\,b}$	$4.72\times10^{6\,c}$	$2.40\times10^{6\,a}$	$2.28\times10^{6\,a}$	$2.90\times10^{5\,b}$	***
2-propanone, 1-hydroxy-	C3H6O2	116-09-6	735	MS, IHL	$1.22\times10^{5\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	$3.83\times10^{6\,b}$	***
2,3-butanedione	C4H6O2	431-03-8	634	MS, IHL, LRI	0.00×00	$6.38\times10^{5\,a}$	$2.36\times10^{6\,b}$	$2.14\times10^{6\,b}$	$4.71\times10^{5\,a}$	$1.73\times10^{5\,a}$	***
2-butanone	C4H8O	78-93-3	640	MS, IHL, LRI	$2.56\times10^{5\,a}$	$1.24\times10^{5\,a}$	$6.72\times10^{5\,b}$	$1.99\times10^{6\,c}$	$5.81\times10^{5\,b}$	0.00×00	***
Acetoin	C4H8O2	513-86-0	779	MS, IHL, LRI	0.00×00	$1.21\times10^{6\,a}$	$9.29\times10^{5\,ab}$	$3.31\times10^{6\,c}$	$1.36\times10^{6\,a}$	0.00×00	***
1-hydroxy-2-butanone	C4H8O2	5077-67-8	819	MS	$2.99\times10^{4\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	ns

1-penten-3-one	C5H8O	1629-58-9	729	MS	$7.93\times10^{4\,a}$	0.00×00	0.00×00	$9.78\times10^{5\,b}$	0.00×00	0.00×00	***
3-penten-2-one	C5H8O	625-33-2	792	MS	0.00×00	$4.19\times10^{4\,a}$	$4.78\times10^{4\,a}$	$6.23\times10^{5\,b}$	$1.76\times10^{5\,c}$	0.00×00	***
3-pentanone	C5H10O	96-22-0	739	MS, LRI	0.00×00	0.00×00	0.00×00	0.00×00	$3.67\times10^{5\,a}$	0.00×00	*
Methyl isobutyl ketone	C6H12O	108-10-1	785	MS, IHL	0.00×00	0.00×00	$1.48\times10^{5\,a}$	0.00×00	0.00×00	0.00×00	***
Diacetone alcohol	C6H12O2	123-42-2	916	MS, IHL	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$2.52\times10^{5\,a}$	***
1H-pyrrole-2,5-dione, 3-ethyl-4-methyl-	C7H9NO2	20189-42-8	1347	MS	$1.21\times10^{4\,a}$	$1.25\times10^{5\,b}$	0.00×00	$7.45\times10^{4\,c}$	$8.80\times10^{4\,c}$	$2.69\times10^{4\,a}$	***
2-heptanone	C7H14O	110-43-0	938	MS, IHL, LRI	0.00×00	0.00×00	$8.89\times10^{4\,a}$	0.00×00	$2.75\times10^{5\,b}$	0.00×00	***
3-heptanone	C7H14O	106-35-4	930	MS	0.00×00	$1.13\times10^{5\,a}$	$3.13\times10^{5\,b}$	0.00×00	0.00×00	0.00×00	***
Acetophenone	C8H8O	98-86-2	1149	MS, IHL	$1.60\times10^{4\,a}$	0.00×00	$2.10\times10^{4\,a}$	$6.98\times10^{4\,b}$	0.00×00	$1.85\times10^{4\,a}$	***
3,5-octadien-2-one	C8H12O	38284-27-4	1137	MS	$3.47\times10^{5\,a}$	$9.78\times10^{4\,b}$	$1.03\times10^{5\,b}$	$2.91\times10^{6\,c}$	$5.73\times10^{5\,d}$	0.00×00	***
3,5-octadien-2-one, (E,E)-	C8H12O	30086-02-3	1119	MS	0.00×00	0.00×00	$2.17\times10^{4\,a}$	0.00×00	0.00×00	0.00×00	ns
5-hepten-2-one, 6-methyl-	C8H14O	110-93-0	1039	MS, IHL, LRI	0.00×00	0.00×00	0.00×00	$7.82\times10^{5\,a}$	0.00×00	0.00×00	***
Isophorone	C9H14O	78-59-1	1123	MS	$2.86\times10^{5\,a}$	0.00×00	0.00×00	$3.50\times10^{5\,b}$	0.00×00	0.00×00	***
alpha-Ionone	C13H20O	127-41-3	1526	MS	0.00×00	0.00×00	$1.94\times10^{4\,a}$	$1.12\times10^{6\,b}$	0.00×00	0.00×00	***
trans-beta-ionone	C13H20O	79-77-6	1584	MS	$8.94\times10^{4\,a}$	$2.30\times10^{4\,b}$	$3.37\times10^{4\,b}$	$4.90\times10^{5\,c}$	$2.91\times10^{5\,d}$	0.00×00	***
LACTONES											
2(5H)-furanone	C4H4O2	497-23-4	1034	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$2.19\times10^{5\,a}$	***
Butyrolactone	C4H6O2	96-48-0	1027	MS, IHL, LRI	0.00×00	$4.63\times10^{5\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	***
2(3H)-furanone, 5-ethyldihydro-	C6H10O2	695-06-7	1174	MS	0.00×00	$1.64\times10^{5\,a}$	0.00×00	$1.77\times10^{5\,a}$	$2.70\times10^{5\,b}$	$4.84\times10^{4\,c}$	***
PYRAZINES											
Pyrazine	C4H4N2	290-37-9	775	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$4.46\times10^{4\,a}$	***
Pyrazine, methyl-	C5H6N2	109-08-0	867	MS, LRI	0.00×00	0.00×00	0.00×00	$1.26\times10^{5\,a}$	0.00×00	$4.32\times10^{4\,b}$	***

Pyrazine, 2,3-dimethyl-	C6H8N2	5910-89-4	966	MS, IHL	0.00×00	0.00×00	0.00×00	$2.06\times10^{4\,a}$	0.00×00	0.00×00	ns
Pyrazine, 2,6-dimethyl-	C6H8N2	108-50-9	955	MS, LRI	0.00×00	0.00×00	0.00×00	$4.82\times10^{5\,a}$	0.00×00	0.00×00	***
Pyrazine, trimethyl-	C7H10N2	14667-55-1	1047	MS, IHL	0.00×00	0.00×00	0.00×00	$2.42\times10^{5\,a}$	0.00×00	0.00×00	***
Pyrazine, tetramethyl-	C8H12N2	1124-11-4	1125	MS, IHL, LRI	0.00×00	$1.03\times10^{5\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	***
SULFUR COMPOUNDS											
Methanethiol	CH4S	74-93-1	412	MS, IHL, LRI	$1.04\times10^{4\,a}$	$4.02\times10^{3\,b}$	$1.98\times10^{4\,c}$	$4.95\times10^{4\,d}$	$7.55\times10^{3\,a}$	0.00×00	***
Dimethyl sulfide	C2H6S	75-18-3	542	MS, IHL, LRI	$2.59\times10^{4\,a}$	0.00×00	$5.20\times10^{6\,b}$	$2.06\times10^{5\,a}$	$1.33\times10^{5\,a}$	0.00×00	***
Dimethyl disulfide	C2H6S2	624-92-0	782	MS, LRI	0.00×00	0.00×00	$7.53\times10^{3\,a}$	$9.31\times10^{4\,b}$	$9.12\times10^{4\,b}$	0.00×00	***
TERPENES											
o-cymene	C10H14	527-84-4	1062	MS	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	$1.02\times10^{4\,a}$	***
D-limonene	C10H16	5989-27-5	1058	MS, IHL, LRI	$7.31\times10^{5\,a}$	0.00×00	$1.57\times10^{5\,b}$	$8.26\times10^{5\,c}$	0.00×00	$3.11\times10^{4\,d}$	***
3-carene	C10H16	13466-78-9	1035	MS, IHL, LRI	0.00×00	$2.25\times10^{4\,a}$	$1.20\times10^{4\,a}$	0.00×00	$1.55\times10^{4\,a}$	$9.94\times10^{3\,a}$	ns
alpha-pinene	C10H16	80-56-8	959	MS, IHL	$3.69\times10^{4\,a}$	0.00×00	$3.65\times10^{4\,a}$	$4.35\times10^{4\,a}$	$4.24\times10^{4\,a}$	0.00×00	***
beta-cyclocitral	C10H16O	432-25-7	1302	MS	$7.58\times10^{4\,a}$	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	***
OTHERS											
Trichloromethane	CHCl3	67-66-3	658	MS, IHL	$1.30\times10^{4\,ab}$	0.00×00	$1.91\times10^{4\,ac}$	$2.93\times10^{4\,d}$	$2.37\times10^{4\,cd}$	$8.55\times10^{3\,b}$	***
2,4-di-tert-butylphenol	C14H22O	96-76-4	1603	MS, IHL	$8.16\times10^{3\,a}$	$1.04\times10^{4\,a}$	$2.75\times10^{4\,a}$	0.00×00	$2.31\times10^{4\,a}$	$5.88\times10^{5\,b}$	***

Levels of volatile compounds are expressed as abundances (mean values from 3 determinations from each seaweed). RT and RI, retention time and retention index on a DB-624 UI column; IM, identification method; MS, spectra comparison using NIST mass spectral database; IHL, in-house library created using authentic compounds with target and qualifier ions and linear retention indices for each compound; LRI, RI agree with literature values. ns, not significant; *, P <0.05; **, P <0.01; ***, P <0.001, significance of seaweed species according to ANOVA. $^{a\text{-}d}$, means in the same row bearing different superscript letter denote statistical significant differences (P <0.05).

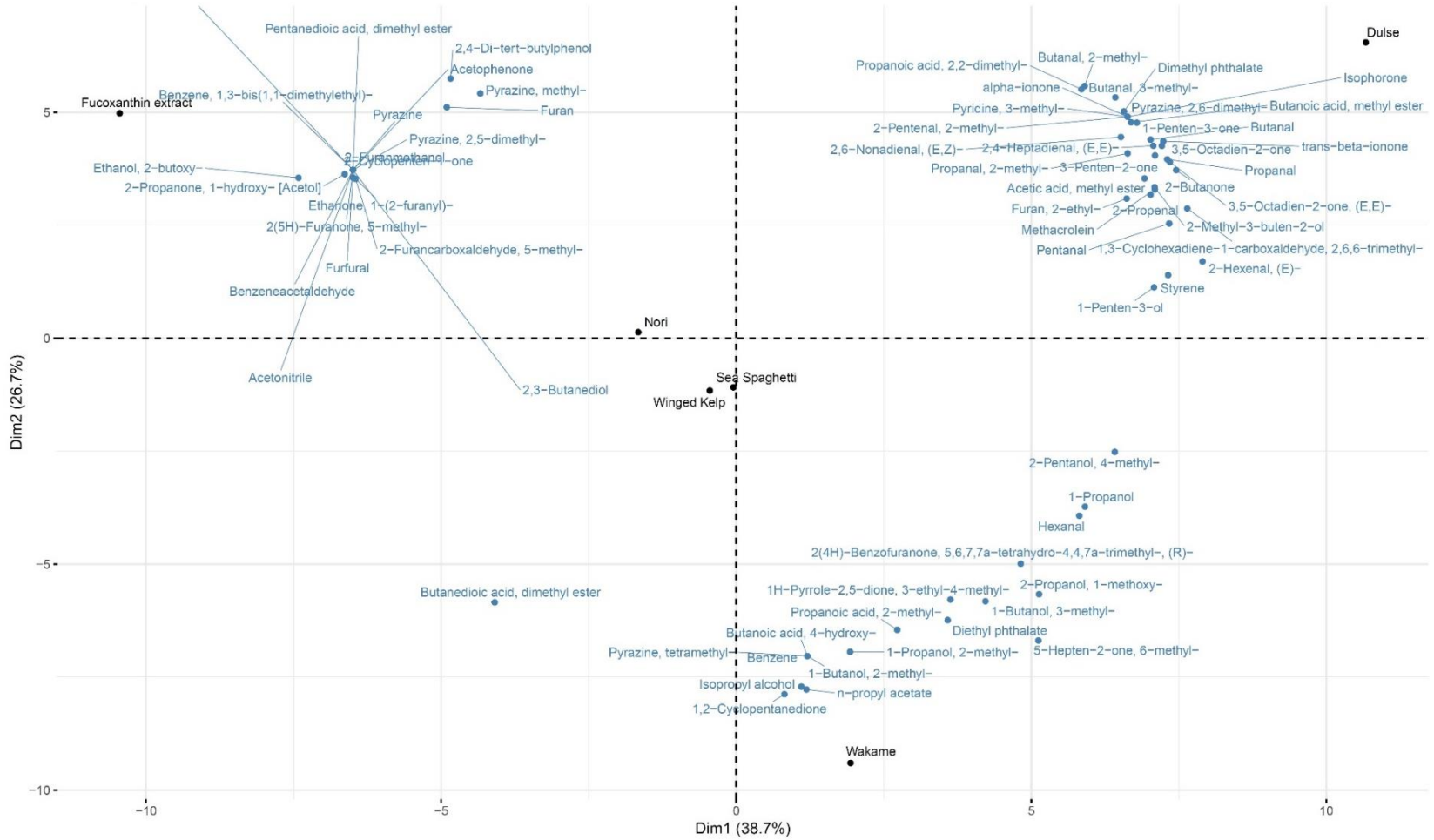


Figure S-3.2. Principal component analysis (PCA) biplot of *H.elongata* (Sea Spaghetti), *U.pinnatifida* (Wakame), *A.esculenta* (Winged Kelp), *L. japonica* fucoxanthin extract, *P.umbilicalis* (Nori) and *P.palmata* (Dulse) samples and the volatile compounds contributing to the differences between the samples, identified by TD GC-MS.

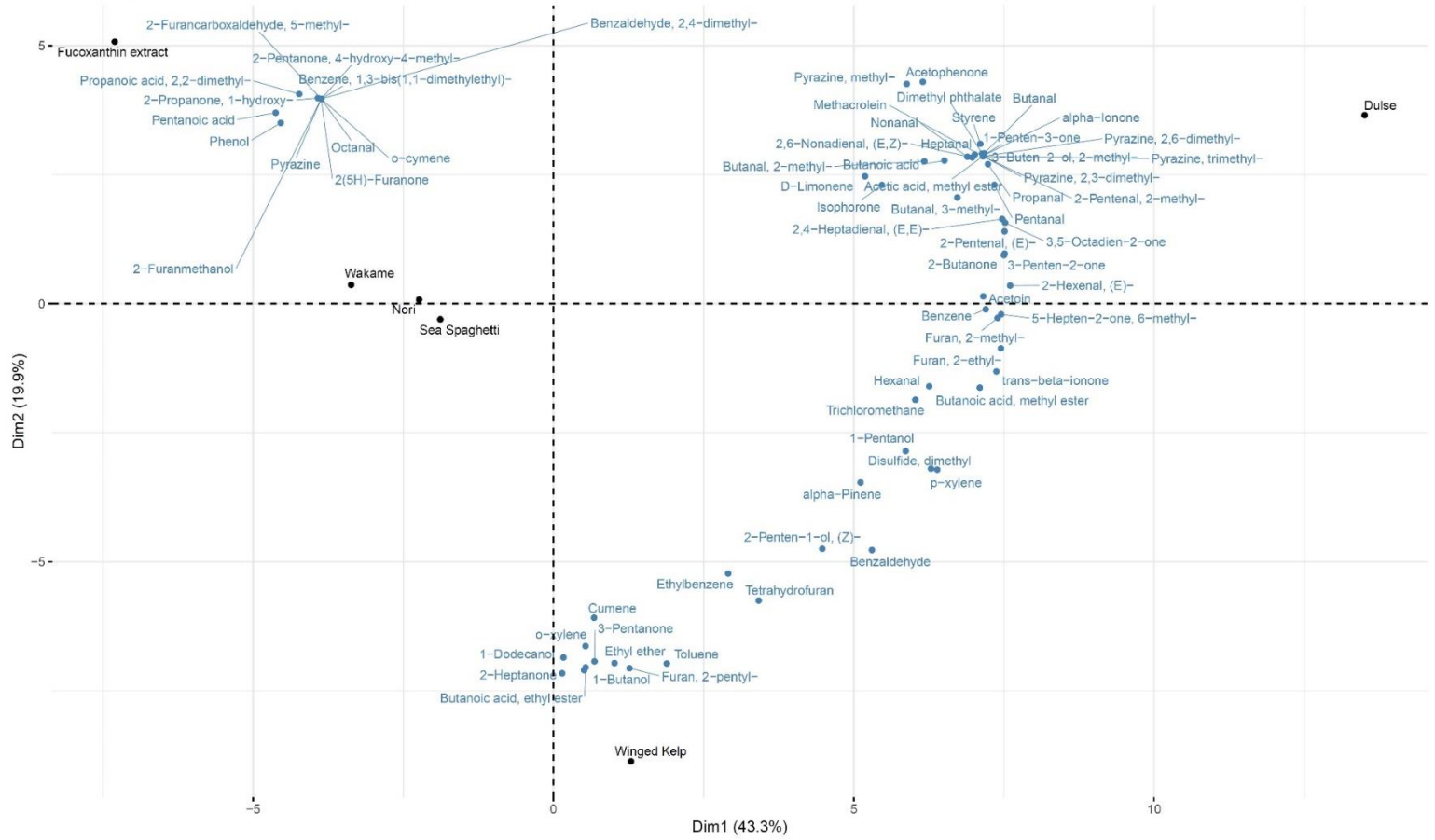


Figure S-3.3. Principal component analysis (PCA) biplot of *H.elongata* (Sea Spaghetti), *U.pinnatifida* (Wakame), *A.esculenta* (Winged Kelp), *L. japonica* fucoxanthin extract, *P.umbilicalis* (Nori) and *P.palmata* (Dulse) samples and the volatile compounds contributing to the differences between the samples, identified by HS-SPME GC-MS.

Chapter 4. Effect of salt reduction and inclusion of 1 % edible seaweed on the chemical, sensory and volatile component profile of reformulated frankfurters.

Table S-4.1. Volatile compounds identified in the different frankfurter formulations with edible seaweeds, after TD extraction.

Compound	Formula	CAS#	RI	IM	Control	*H. elongata*	*U. pinnatifida*	*P. umbilicalis*	*P. palmata*
ALCOHOLS									
Isopropyl Alcohol	C3H8O	67-63-0	540	MS	6.55×10^{5}	7.42×10^{5}	8.90×10^{5}	6.43×10^{5}	1.26×10^{6}
1-Propanol	C3H8O	71-23-8	610	MS, IHL, LRI	7.08×10^{5}	5.04×10^{5}	1.85×10^{5}	2.14×10^{5}	2.93×10^{5}
3-Buten-2-ol, 2-methyl-	C5H10O	115-18-4	658	MS, LRI	1.98×10^{5}	1.53×10^{5}	1.57×10^{5}	1.42×10^{5}	2.06×10^{5}
1-Propanol, 2-methyl-	C4H10O	78-83-1	678	MS	7.85×10^{4}	8.99×10^{4}	8.95×10^{4}	1.22×10^{5}	9.81×10^{4}
1-Butanol	C4H10O	71-36-3	715	MS, IHL	2.08×10^{5}	2.50×10^{5}	2.96×10^{5}	2.98×10^{5}	2.30×10^{5}
2-Propanol, 1-methoxy-	C4H10O2	107-98-2	718	MS, IHL	4.21×10^{6}	1.85×10^{6}	1.01×10^{6}	1.04×10^{6}	1.26×10^{6}
1-Penten-3-ol	C5H10O	616-25-1	732	MS	2.86×10^{5}	9.95×10^{5}	7.35×10^{5}	8.63×10^{5}	1.65×10^{6}
Isopropyl Alcohol	C3H8O	67-63-0	791	MS	1.22×10^{5}	6.64×10^{4}	0.00×00	9.24×10^{4}	0.00×00
2-Pentanol, 4-methyl-	C6H14O	108-11-2	805	MS	6.93×10^{6}	7.52×10^{6}	7.47×10^{6}	7.71×10^{6}	7.66×10^{6}
1-Pentanol	C5H12O	71-41-0	817	MS, IHL, LRI	3.30×10^{5}	3.63×10^{5}	4.27×10^{5}	5.06×10^{5}	4.16×10^{5}
1-Hexanol	C6H14O	111-27-3	918	MS, IHL, LRI	7.96×10^{5}	8.64×10^{5}	8.37×10^{5}	8.23×10^{5}	8.76×10^{5}
3-Heptanol	C7H16O	589-82-2	940	MS	1.97×10^{4}	0.00×00	4.63×10^{4}	5.91×10^{4}	3.58×10^{4}
Ethanol, 2-butoxy-	C6H14O2	111-76-2	957	MS, LRI	1.14×10^{5}	1.24×10^{5}	1.14×10^{5}	1.40×10^{5}	1.25×10^{5}
1-Octen-3-ol	C8H16O	3391-86-4	1027	MS, IHL, LRI	5.25×10^{5}	1.19×10^{6}	1.62×10^{6}	1.32×10^{6}	7.23×10^{5}
1-Hexanol, 2-ethyl-	C8H18O	104-76-7	1080	MS, IHL, LRI	3.78×10^{5}	4.11×10^{5}	3.47×10^{5}	4.08×10^{5}	4.91×10^{5}
1-Octanol	C8H18O	1568-20-3	1121	MS	1.03×10^{5}	1.03×10^{5}	1.14×10^{5}	8.73×10^{4}	8.73×10^{4}
4-Thujanol	C10H18O	546-79-2	1131	MS	8.29×10^{5}	5.81×10^{5}	4.25×10^{5}	5.68×10^{5}	5.09×10^{5}
trans-4-Thujanol	C10H18O	17699-16-0	1155	MS	1.44×10^{6}	1.78×10^{6}	1.63×10^{6}	1.62×10^{6}	1.69×10^{6}
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	C10H18O	29803-82-5	1189	MS	4.62×10^{4}	2.49×10^{4}	1.62×10^{4}	3.07×10^{4}	1.51×10^{4}

Fenchol	C10H18O	1632-73-1	1192	MS	2.42×10^{5}	2.46×10^{5}	2.28×10^{5}	2.18×10^{5}	2.31×10^{5}
Terpinen-4-ol	C10H18O	562-74-3	1245	MS, LRI	3.71×10^{6}	4.36×10^{6}	3.99×10^{6}	4.43×10^{6}	4.53×10^{6}
ALDEHYDES									
Propanal	C3H6O	123-38-6	526	MS, IHL, LRI	1.51×10^{5}	1.80×10^{5}	2.42×10^{5}	1.47×10^{5}	6.42×10^{5}
Propanal, 2-methyl-	C4H8O	78-84-2	592	MS, IHL, LRI	6.93×10^{5}	4.09×10^{5}	4.54×10^{5}	4.48×10^{5}	5.99×10^{5}
Methacrolein	C4H6O	78-85-3	604	MS, IHL, LRI	6.82×10^{4}	4.81×10^{4}	7.30×10^{4}	7.52×10^{4}	9.41×10^{4}
Butanal	C4H8O	123-72-8	628	MS, LRI	6.11×10^{4}	5.94×10^{4}	9.19×10^{4}	5.93×10^{4}	1.79×10^{5}
Butanal, 3-methyl-	C5H10O	590-86-3	693	MS, IHL, LRI	4.65×10^{5}	4.48×10^{5}	6.94×10^{5}	5.28×10^{5}	7.31×10^{5}
Pentanal	C5H10O	110-62-3	737	MS, IHL, LRI	1.03×10^{6}	8.39×10^{5}	1.30×10^{6}	1.28×10^{6}	1.09×10^{6}
Hexanal	C6H12O	66-25-1	842	MS, IHL, LRI	2.06×10^{6}	2.51×10^{6}	2.75×10^{6}	3.01×10^{6}	1.87×10^{6}
Heptanal	C7H14O	111-71-7	946	MS, IHL, LRI	4.70×10^{5}	3.83×10^{5}	2.91×10^{5}	2.81×10^{5}	3.46×10^{5}
Methional	C4H8OS	3268-49-3	976	MS, LRI	1.22×10^{5}	4.37×10^{4}	4.74×10^{4}	5.05×10^{4}	6.99×10^{4}
2-Heptenal, (Z)-	C7H12O	57266-86-1	1017	MS	1.87×10^{5}	1.81×10^{5}	6.61×10^{5}	3.40×10^{5}	2.21×10^{5}
Octanal	C8H16O	124-13-0	1050	MS, IHL, LRI	3.07×10^{5}	1.52×10^{5}	1.95×10^{5}	1.69×10^{5}	2.06×10^{5}
Benzeneacetaldehyde	C8H8O	122-78-1	1123	MS, IHL, LRI	1.87×10^{5}	1.18×10^{5}	2.47×10^{5}	2.52×10^{5}	2.49×10^{5}
Nonanal	C9H18O	124-19-6	1154	MS, IHL, LRI	1.81×10^{6}	1.40×10^{6}	9.15×10^{5}	8.79×10^{5}	1.39×10^{6}
2-Nonenal, (E)-	C9H16O	18829-56-6	1228	MS	1.14×10^{4}	4.98×10^{4}	3.81×10^{4}	3.87×10^{4}	1.37×10^{4}
Decanal	C10H20O	112-31-2	1258	MS, IHL, LRI	1.62×10^{5}	1.34×10^{5}	1.05×10^{5}	1.47×10^{5}	2.00×10^{5}
2-Decenal, (E)-	C10H18O	3913-81-3	1334	MS	1.07×10^{5}	9.85×10^{4}	1.37×10^{5}	9.13×10^{4}	7.22×10^{4}
2-Undecenal	C11H20O	2463-77-6	1439	MS	4.34×10^{4}	2.56×10^{4}	0.00×00	0.00×00	0.00×00
ESTERS / ETHERS									
Ethyl ether	C4H10O	60-29-7	514	MS, IHL	0.00×00	3.34×10^{4}	3.09×10^{4}	3.18×10^{4}	3.42×10^{4}
Butanoic acid, methyl ester	C5H10O2	623-42-7	751	MS, IHL, LRI	4.19×10^{5}	3.20×10^{5}	1.45×10^{4}	3.66×10^{5}	0.00×00
Methyl valerate	C6H12O2	624-24-8	853	MS	2.57×10^{4}	1.91×10^{4}	0.00×00	3.11×10^{4}	0.00×00
Hexanoic acid, methyl ester	C7H14O2	106-70-7	954	MS	4.13×10^{5}	3.03×10^{5}	1.40×10^{4}	3.55×10^{5}	0.00×00
Octanoic acid, methyl ester	C9H18O2	111-11-5	1156	MS, IHL, LRI	5.28×10^{4}	3.48×10^{4}	0.00×00	4.25×10^{4}	0.00×00
Bornyl acetate	C12H20O2	76-49-3	1355	MS, LRI	1.20×10^{5}	1.70×10^{5}	1.51×10^{5}	1.72×10^{5}	1.69×10^{5}
alpha-Terpinyl acetate	C12H20O2	80-26-2	1409	MS	2.90×10^{4}	1.29×10^{4}	1.49×10^{4}	2.75×10^{4}	2.53×10^{4}

Diethyl Phthalate	C12H14O4	84-66-2	1702	MS	2.64×10^{5}	3.32×10^{5}	1.51×10^{5}	1.71×10^{5}	2.23×10^{5}
Eucalyptol	C10H18O	470-82-6	1072	MS, IHL, LRI	3.18×10^{5}	3.63×10^{5}	3.35×10^{5}	2.94×10^{5}	3.91×10^{5}
KETONES									
Acetone	C3H6O	67-64-1	531	MS, IHL, LRI	3.74×10^{6}	2.42×10^{6}	8.00×10^{5}	1.57×10^{6}	8.83×10^{5}
2,3-Butanedione	C4H6O2	431-03-8	631	MS, IHL, LRI	1.25×10^{6}	1.59×10^{6}	1.54×10^{6}	1.67×10^{6}	1.42×10^{6}
2-Butanone	C4H8O	78-93-3	638	MS, IHL, LRI	2.58×10^{6}	1.33×10^{6}	1.34×10^{6}	1.54×10^{6}	2.24×10^{6}
2-Pentanone	C5H10O	107-87-9	731	MS, IHL, LRI	1.88×10^{5}	6.32×10^{4}	1.46×10^{5}	2.03×10^{5}	0.00×00
Acetoin	C4H8O2	513-86-0	779	MS, IHL, LRI	3.46×10^{6}	3.55×10^{6}	3.46×10^{6}	4.36×10^{6}	3.10×10^{6}
Methyl Isobutyl Ketone	C6H12O	108-10-1	783	MS, IHL	3.01×10^{5}	6.27×10^{5}	4.64×10^{5}	1.01×10^{6}	4.68×10^{5}
2-Hexanone	C6H12O	591-78-6	835	MS, IHL, LRI	2.03×10^{4}	3.04×10^{4}	3.50×10^{4}	6.03×10^{4}	5.46×10^{4}
3-Heptanone	C7H14O	106-35-4	930	MS	7.94×10^{4}	7.34×10^{4}	8.16×10^{4}	9.98×10^{4}	8.93×10^{4}
2-Heptanone	C7H14O	110-43-0	937	MS, IHL, LRI	1.05×10^{5}	1.14×10^{5}	1.30×10^{5}	1.22×10^{5}	1.24×10^{5}
5-Hepten-2-one, 6-methyl-	C8H14O	110-93-0	1036	MS, IHL, LRI	5.98×10^{5}	8.06×10^{5}	7.86×10^{5}	7.23×10^{5}	7.65×10^{5}
MONOTERPENES									
Camphene	C10H16	79-92-5	983	MS, IHL, LRI	8.32×10^{5}	2.46×10^{6}	2.53×10^{6}	2.39×10^{6}	2.37×10^{6}
beta-Pinene	C10H16	127-91-3	1003	MS	4.92×10^{6}	8.78×10^{6}	6.28×10^{6}	5.20×10^{6}	6.75×10^{6}
alpha-Phellandrene	C10H16	99-83-2	1034	MS	3.27×10^{6}	4.76×10^{6}	5.17×10^{6}	4.82×10^{6}	5.79×10^{6}
3-Carene	C10H16	13466-78-9	1046	MS, IHL, LRI	5.27×10^{6}	6.57×10^{6}	6.70×10^{6}	6.48×10^{6}	6.91×10^{6}
o-Cymene	C10H14	527-84-4	1061	MS	6.64×10^{6}	1.02×10^{7}	1.00×10^{7}	1.06×10^{7}	1.01×10^{7}
beta-Phellandrene	C10H16	555-10-2	1066	MS, LRI	5.66×10^{6}	8.23×10^{6}	7.93×10^{6}	7.78×10^{6}	8.61×10^{6}
gamma-Terpinene	C10H16	99-85-4	1085	MS	6.70×10^{6}	7.31×10^{6}	7.07×10^{6}	7.37×10^{6}	7.32×10^{6}
MONOTERPENOIDS									
alpha-Thujene	C10H16	2867-05-2.	943	MS	4.61×10^{6}	7.94×10^{6}	6.77×10^{6}	5.31×10^{6}	7.11×10^{6}
alpha-Pinene	C10H16	80-56-8	960	MS, IHL	4.56×10^{6}	7.20×10^{6}	5.79×10^{6}	3.74×10^{6}	5.69×10^{6}
alpha-Fenchene	C10H16	471-84-1	967	MS	0.00×00	6.97×10^{4}	1.44×10^{5}	1.36×10^{5}	9.95×10^{4}
PHENOLS									
Prenol	C5H10O	556-82-1	827	MS, LRI	2.80×10^{5}	2.72×10^{5}	2.47×10^{5}	2.78×10^{5}	3.42×10^{5}
Phenol	C6H6O	108-95-2	1098	MS	9.51×10^{4}	9.91×10^{4}	5.85×10^{4}	6.69×10^{4}	7.24×10^{4}
2,4-Di-tert-butylphenol	C14H22O	96-76-4	1603	MS, IHL	2.64×10^{6}	3.13×10^{6}	3.13×10^{6}	3.74×10^{6}	3.21×10^{6}

PHENYLPROPANES									
Safrole	C10H10O2	94-59-7	1360	MS	3.70×10^{5}	4.63×10^{5}	4.17×10^{5}	4.69×10^{5}	4.75×10^{5}
Methyleugenol	C11H14O2	93-15-2	1468	MS	1.40×10^{5}	1.76×10^{5}	1.60×10^{5}	1.90×10^{5}	1.80×10^{5}
Myristicin	C11H12O3	607-91-0	1608	MS	3.98×10^{5}	5.03×10^{5}	4.71×10^{5}	5.68×10^{5}	5.17×10^{5}
Elemicin	C12H16O3	487-11-6	1624	MS	2.90×10^{5}	3.35×10^{5}	3.68×10^{5}	5.34×10^{5}	3.08×10^{5}
SESQUITERPENES									
alpha-Cubebene	C15H24	17699-14-8	1395	MS	6.18×10^{4}	9.69×10^{4}	8.42×10^{4}	9.01×10^{4}	9.84×10^{4}
Copaene	C15H24	3856-25-5	1432	MS	3.09×10^{5}	4.53×10^{5}	4.29×10^{5}	4.41×10^{5}	4.50×10^{5}
Caryophyllene	C15H24	87-44-5	1491	MS, LRI	5.82×10^{5}	8.56×10^{5}	7.64×10^{5}	8.50×10^{5}	8.64×10^{5}
beta-Bisabolene	C15H24	495-61-4	1535	MS	2.13×10^{4}	0.00×00	0.00×00	7.88×10^{4}	9.33×10^{4}
beta-Sesquiphellandrene	C15H24	20307-83-9	1571	MS	9.32×10^{4}	1.32×10^{5}	1.23×10^{5}	1.54×10^{5}	1.65×10^{5}
SULPHUR COMPOUNDS									
Sulfur dioxide	O2S	7446-09-5	320	MS	1.92×10^{6}	1.95×10^{6}	2.25×10^{6}	2.82×10^{6}	2.58×10^{6}
Carbon disulfide	CS2	75-15-0	546	MS, IHL, LRI	1.51×10^{6}	1.75×10^{6}	1.21×10^{6}	1.26×10^{6}	1.49×10^{6}
Disulfide, dimethyl	C2H6S2	624-92-0	781	MS, LRI	5.63×10^{4}	5.78×10^{4}	8.65×10^{4}	5.81×10^{4}	9.06×10^{4}
Dimethyl trisulfide	C2H6S3	3658-80-8	1027	MS	4.33×10^{4}	2.32×10^{4}	1.94×10^{4}	4.68×10^{4}	8.04×10^{4}
TERPENES									
D-Limonene	C10H16	5989-27-5	1058	MS, IHL, LRI	3.59×10^{6}	4.75×10^{6}	4.41×10^{6}	4.38×10^{6}	4.60×10^{6}
Terpinolene	C10H16	586-62-9	1118	MS	3.32×10^{6}	4.68×10^{6}	4.69×10^{6}	4.23×10^{6}	4.74×10^{6}
(+)-2-Bornanone	C10H16O	464-49-3	1236	MS	3.16×10^{5}	4.25×10^{5}	3.85×10^{5}	4.42×10^{5}	4.18×10^{5}
endo-Borneol	C10H18O	507-70-0	1255	MS	1.77×10^{6}	1.75×10^{6}	1.74×10^{6}	1.65×10^{6}	1.72×10^{6}
alpha-Terpineol	C10H18O	98-55-5	1264	MS, IHL, LRI	1.19×10^{6}	1.33×10^{6}	1.23×10^{6}	1.32×10^{6}	1.29×10^{6}
alpha-Curcumene	C15H22	644-30-4	1530	MS	2.42×10^{5}	3.09×10^{5}	2.80×10^{5}	3.20×10^{5}	3.28×10^{5}
OTHERS									
Toluene	C7H8	108-88-3	796	MS	1.93×10^{5}	2.25×10^{5}	2.94×10^{5}	3.84×10^{5}	2.83×10^{5}
Furan, 2-pentyl-	C9H14O	3777-69-3	1016	MS, IHL, LRI	7.92×10^{4}	1.37×10^{5}	1.34×10^{5}	1.08×10^{5}	1.29×10^{5}
2(3H)-Furanone, 5-butyldihydro-	C8H14O2	104-50-7	1388	MS	5.21×10^{4}	5.90×10^{4}	5.13×10^{4}	5.77×10^{4}	6.15×10^{4}
Pyrrole	C4H5N	109-97-7	836	MS	3.56×10^{4}	0.00×00	0.00×00	0.00×00	1.14×10^{4}

Pyrazine, tetramethyl-	C8H12N2	1124-11-4	1127	MS, IHL, LRI	1.34×10^5	1.47×10^5	1.61×10^5	1.02×10^5	1.54×10^5

Levels of volatile compounds are expressed as abundances (mean values from 3 determinations from each sample). RI, retention index on a DB-624 UI column; IM, identification method; MS, spectra comparison using NIST mass spectral database; IHL, in-house library created using authentic compounds with target and qualifier ions and linear RI for each compound; LRI, RI agree with literature values.

Chapter 6. High capacity sorptive extraction and thermal desorption gas chromatography-olfactometry for the screening of lipid-oxidised aromas of seaweed-containing raw beef patties.

Table S-6.1. Gas content of MAP at the start and end of storage, and fat and moisture of control and reformulated beef patties.

		Baseline	**End**
% Gas	**O_2**	76.8	76.1
	CO_2	21.8	21.8
% Fat [a]		13.6	-
% Moisture [a]		66.57	-

[a] Mean of 3 measurements/sample

Table S-6.2. Full volatile profile of control and reformulated beef patties after GC-MS/O analysis across storage time.

			Control			Sea Spaghetti			Nori		
Compound	**RT**	**RI**	**T0**	**T7**	**T14**	**T0**	**T7**	**T14**	**T0**	**T7**	**T14**
Ethyl ether	2.612	631	5.86×10^{5}	4.84×10^{5}	3.73×10^{5}	5.04×10^{5}	4.12×10^{5}	2.39×10^{5}	4.84×10^{5}	4.85×10^{5}	2.85×10^{5}
Acetone	2.836	648	6.36×10^{4}	7.52×10^{4}	1.54×10^{5}	8.52×10^{4}	2.81×10^{5}	1.25×10^{5}	1.18×10^{5}	1.63×10^{5}	2.24×10^{5}
n-Hexane	3.672	705	7.21×10^{4}	9.03×10^{4}	3.80×10^{4}	3.95×10^{4}	7.02×10^{4}	2.56×10^{4}	1.72×10^{5}	7.44×10^{4}	4.80×10^{4}
Butanal	4.246	732	2.24×10^{5}	0.00×00	0.00×00	1.80×10^{5}	8.76×10^{4}	0.00×00	3.63×10^{5}	6.77×10^{4}	0.00×00
2,3-Butanedione	4.338	736	1.03×10^{6}	1.19×10^{7}	1.72×10^{7}	1.09×10^{6}	7.47×10^{6}	8.27×10^{6}	6.63×10^{5}	1.44×10^{7}	1.14×10^{7}
2-Butanone	4.471	742	0.00×00	0.00×00	0.00×00	0.00×00	8.62×10^{5}	3.30×10^{6}	0.00×00	0.00×00	4.77×10^{6}
Benzene	5.441	788	7.08×10^{4}	1.39×10^{5}	2.40×10^{5}	2.72×10^{5}	2.10×10^{5}	1.80×10^{5}	7.96×10^{4}	2.65×10^{5}	1.98×10^{5}
Butanal, 3-methyl-	5.652	797	1.53×10^{6}	6.99×10^{6}	6.48×10^{6}	7.69×10^{5}	3.02×10^{6}	1.67×10^{6}	1.38×10^{6}	3.95×10^{6}	1.89×10^{6}
Heptane	5.833	805	4.30×10^{5}	4.78×10^{5}	3.60×10^{5}	4.04×10^{5}	4.04×10^{5}	2.54×10^{5}	7.73×10^{5}	5.83×10^{5}	4.34×10^{5}
Acetic acid	5.980	811	3.03×10^{5}	6.44×10^{5}	6.80×10^{5}	1.00×10^{6}	1.25×10^{6}	1.15×10^{6}	8.97×10^{5}	9.83×10^{5}	7.98×10^{5}
Furan, 2-ethyl-	6.298	823	1.09×10^{5}	1.53×10^{6}	1.77×10^{6}	6.73×10^{4}	1.06×10^{6}	5.83×10^{5}	8.04×10^{4}	1.43×10^{6}	8.44×10^{5}
1-Butanol	6.337	825	0.00×00	0.00×00	1.49×10^{5}	0.00×00	0.00×00	0.00×00	7.97×10^{4}	2.20×10^{4}	0.00×00
2-Pentanone	6.612	835	0.00×00	1.15×10^{4}	0.00×00	0.00×00	0.00×00	2.45×10^{4}	8.20×10^{4}	1.65×10^{4}	4.31×10^{4}
Pentanal	6.746	840	9.24×10^{5}	0.00×00	9.22×10^{5}	8.11×10^{5}	9.87×10^{5}	5.33×10^{5}	1.68×10^{6}	1.59×10^{6}	1.09×10^{6}
2,3-Pentanedione	6.818	843	0.00×00	7.32×10^{5}	3.94×10^{5}	0.00×00	6.82×10^{4}	1.15×10^{5}	0.00×00	1.53×10^{5}	1.72×10^{5}
Butanoic acid, methyl ester	7.151	856	5.94×10^{3}	1.91×10^{4}	2.39×10^{4}	1.48×10^{4}	1.65×10^{4}	0.00×00	1.78×10^{4}	2.17×10^{4}	3.75×10^{3}

Acetoin	8.035	891	3.65×10^{6}	1.31×10^{7}	2.04×10^{7}	5.09×10^{6}	2.14×10^{7}	1.96×10^{7}	4.31×10^{6}	2.79×10^{7}	2.53×10^{7}
Octane	8.568	911	8.19×10^{5}	3.17×10^{5}	2.78×10^{5}	5.11×10^{5}	2.98×10^{5}	2.70×10^{5}	9.87×10^{5}	4.15×10^{5}	2.13×10^{5}
2-Pentanol, 4-methyl-	8.822	920	7.84×10^{6}	4.87×10^{6}	4.95×10^{6}	6.93×10^{6}	4.13×10^{6}	2.89×10^{6}	5.93×10^{6}	4.64×10^{6}	2.76×10^{6}
1-Pentanol	9.203	934	1.26×10^{6}	3.54×10^{6}	6.82×10^{6}	3.47×10^{5}	4.04×10^{6}	2.54×10^{6}	4.59×10^{5}	3.93×10^{6}	2.40×10^{6}
2-Hexanone	9.513	946	0.00×00	0.00×00	2.12×10^{4}	2.61×10^{4}	4.17×10^{3}	1.91×10^{4}	9.98×10^{4}	0.00×00	0.00×00
Hexanal	9.683	952	4.13×10^{6}	9.64×10^{6}	5.47×10^{6}	2.22×10^{6}	2.09×10^{6}	8.10×10^{5}	3.53×10^{6}	2.72×10^{6}	1.22×10^{6}
Butanoic acid	11.080	1003	7.74×10^{5}	5.72×10^{5}	4.18×10^{5}	1.42×10^{6}	1.53×10^{6}	9.61×10^{5}	2.78×10^{6}	1.50×10^{6}	1.40×10^{6}
2,3-Butanediol	11.156	1007	0.00×00	0.00×00	0.00×00	0.00×00	3.33×10^{5}	3.60×10^{5}	0.00×00	2.46×10^{5}	2.39×10^{5}
Furfural	11.426	1016	0.00×00	4.82×10^{4}	2.45×10^{4}	4.07×10^{4}	2.84×10^{4}	1.91×10^{4}	1.12×10^{4}	4.97×10^{4}	3.90×10^{4}
Nonane	11.475	1019	6.73×10^{4}	4.05×10^{4}	3.44×10^{4}	5.52×10^{4}	3.87×10^{4}	4.21×10^{4}	1.12×10^{5}	6.48×10^{4}	4.48×10^{4}
2-Hexenal, (E)-	11.645	1025	5.92×10^{4}	2.19×10^{5}	2.75×10^{5}	1.88×10^{4}	1.22×10^{5}	2.02×10^{5}	8.94×10^{4}	1.96×10^{5}	1.37×10^{5}
2-n-Butyl furan	11.743	1029	1.36×10^{4}	4.72×10^{4}	1.15×10^{5}	2.13×10^{4}	8.27×10^{4}	7.12×10^{4}	5.35×10^{4}	7.22×10^{4}	7.70×10^{4}
Hexane, 1-chloro-	11.825	1002	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00
1-Hexanol	12.106	1043	1.07×10^{6}	1.38×10^{7}	8.78×10^{6}	6.05×10^{5}	1.55×10^{7}	8.49×10^{6}	6.54×10^{5}	9.89×10^{6}	7.94×10^{6}
2-Heptanone	12.469	1056	2.58×10^{5}	8.14×10^{5}	1.23×10^{6}	2.94×10^{5}	9.91×10^{5}	9.51×10^{5}	5.94×10^{5}	8.41×10^{5}	1.07×10^{6}
Heptanal	12.666	1064	3.34×10^{6}	3.84×10^{6}	4.22×10^{6}	2.69×10^{6}	1.94×10^{6}	1.24×10^{6}	5.48×10^{6}	3.10×10^{6}	1.50×10^{6}
Hexanoic acid, methyl ester	12.965	1075	8.06×10^{5}	2.31×10^{6}	5.79×10^{6}	5.65×10^{5}	3.06×10^{6}	2.19×10^{6}	1.89×10^{5}	2.89×10^{6}	2.09×10^{6}
Cyclohexanone	12.977	1075	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	5.24×10^{5}	0.00×00	0.00×00
Butanoic acid, 3-methyl-, 1-methylethyl ester	13.312	1088	2.87×10^{4}	3.88×10^{4}	3.45×10^{4}	1.42×10^{4}	3.29×10^{4}	3.22×10^{4}	1.40×10^{4}	3.91×10^{4}	2.47×10^{4}

3-Heptanone, 2-methyl-	13.669	1102	2.99×10^{7}	2.07×10^{7}	1.99×10^{7}	2.72×10^{7}	1.96×10^{7}	1.86×10^{7}	2.44×10^{7}	2.31×10^{7}	1.73×10^{7}
Pentanoic acid	13.717	1103	1.10×10^{5}	9.13×10^{4}	9.44×10^{4}	1.94×10^{5}	1.75×10^{5}	1.39×10^{5}	7.71×10^{5}	1.86×10^{6}	2.18×10^{5}
2-Heptanone, 6-methyl-	14.301	1126	0.00×00	5.42×10^{4}	1.85×10^{5}	3.55×10^{4}	1.68×10^{5}	1.14×10^{5}	1.25×10^{4}	2.13×10^{5}	2.21×10^{5}
Furan, 2-pentyl-	14.583	1138	3.63×10^{5}	1.55×10^{6}	2.90×10^{6}	5.08×10^{5}	1.76×10^{6}	1.58×10^{6}	7.13×10^{5}	1.47×10^{6}	1.48×10^{6}
2-Heptenal, (Z)-	14.642	1140	2.11×10^{5}	7.59×10^{5}	1.21×10^{6}	2.02×10^{5}	3.81×10^{5}	4.91×10^{5}	2.67×10^{5}	7.86×10^{5}	4.88×10^{5}
4-Octanone	14.682	1142	8.07×10^{4}	3.49×10^{4}	1.09×10^{4}	8.09×10^{4}	1.01×10^{4}	8.97×10^{3}	9.55×10^{4}	3.15×10^{4}	0.00×00
Benzaldehyde	14.898	1150	4.98×10^{5}	1.41×10^{7}	3.28×10^{7}	5.26×10^{5}	2.80×10^{7}	2.32×10^{7}	6.32×10^{5}	2.91×10^{7}	2.56×10^{7}
1-Octen-3-ol	15.067	1157	1.90×10^{6}	4.05×10^{6}	5.56×10^{6}	2.26×10^{6}	5.34×10^{6}	3.80×10^{6}	9.49×10^{5}	4.25×10^{6}	4.78×10^{6}
5-Hepten-2-one, 6-methyl-	15.208	1163	5.01×10^{4}	1.07×10^{5}	1.57×10^{5}	7.92×10^{4}	1.02×10^{5}	9.37×10^{4}	3.57×10^{4}	1.38×10^{5}	1.72×10^{5}
2-Octanone	15.305	1166	5.60×10^{4}	6.54×10^{4}	1.03×10^{5}	8.59×10^{4}	9.03×10^{4}	8.96×10^{4}	2.77×10^{5}	1.31×10^{5}	1.42×10^{5}
Octanal	15.531	1175	3.28×10^{6}	2.72×10^{6}	2.39×10^{6}	3.44×10^{6}	2.10×10^{6}	1.36×10^{6}	6.87×10^{6}	3.40×10^{6}	2.00×10^{6}
Heptanoic acid, methyl ester	15.735	1183	4.32×10^{4}	8.99×10^{4}	3.90×10^{5}	2.97×10^{4}	1.24×10^{5}	1.33×10^{5}	1.41×10^{4}	1.20×10^{5}	1.52×10^{5}
Hexanoic acid	16.291	1206	2.08×10^{6}	2.51×10^{6}	3.22×10^{6}	2.73×10^{6}	3.14×10^{6}	2.61×10^{6}	5.31×10^{6}	3.99×10^{6}	3.70×10^{6}
1-Hexanol, 2-ethyl-	16.457	1212	1.66×10^{5}	1.15×10^{5}	9.67×10^{4}	1.02×10^{5}	9.35×10^{4}	1.10×10^{5}	2.47×10^{5}	6.49×10^{4}	3.33×10^{5}
Octane, 1-chloro-	16.623	1219	5.09×10^{5}	3.08×10^{5}	1.99×10^{5}	2.91×10^{5}	2.47×10^{5}	1.81×10^{5}	2.95×10^{5}	2.77×10^{5}	1.29×10^{5}
Phenol	17.203	1243	6.77×10^{4}	7.38×10^{4}	6.42×10^{4}	1.03×10^{5}	7.22×10^{4}	9.25×10^{4}	1.11×10^{5}	8.63×10^{4}	5.88×10^{4}
Benzeneacetaldehyde	17.207	1246	6.13×10^{4}	2.21×10^{5}	2.57×10^{5}	2.45×10^{4}	4.21×10^{5}	6.35×10^{5}	0.00×00	2.89×10^{5}	5.92×10^{5}
2-Octenal, (E)-	17.409	1252	4.71×10^{5}	8.45×10^{5}	1.24×10^{6}	3.09×10^{5}	4.76×10^{5}	4.59×10^{5}	1.20×10^{6}	9.44×10^{5}	1.07×10^{6}
1-Octanol	17.571	1259	1.25×10^{6}	0.00×00	3.37×10^{6}	1.00×10^{6}	2.10×10^{6}	1.88×10^{6}	1.46×10^{6}	2.09×10^{6}	1.94×10^{6}

3,5-Octadien-2-one, (E,E)-	17.806	1269	3.27×10^{4}	1.11×10^{6}	2.52×10^{5}	2.02×10^{4}	1.64×10^{5}	8.05×10^{4}	0.00×00	1.67×10^{5}	1.55×10^{5}
Acetophenone	17.863	1271	1.25×10^{5}	7.28×10^{4}	7.63×10^{4}	1.28×10^{5}	1.25×10^{5}	4.51×10^{4}	1.50×10^{5}	1.05×10^{5}	8.16×10^{4}
Nonanal	18.248	1287	1.94×10^{7}	1.82×10^{7}	1.53×10^{7}	1.71×10^{7}	1.26×10^{7}	7.75×10^{6}	2.51×10^{7}	1.84×10^{7}	9.75×10^{6}
2,5-Dihydroxybenzaldehyde, 2TMS derivative	18.463	1296	1.11×10^{6}	1.01×10^{6}	9.03×10^{5}	1.04×10^{6}	8.23×10^{5}	1.09×10^{6}	4.15×10^{6}	2.85×10^{6}	2.95×10^{6}
Heptanoic acid	18.614	1304	1.27×10^{6}	8.29×10^{5}	6.95×10^{5}	1.24×10^{6}	9.50×10^{5}	8.44×10^{5}	3.26×10^{6}	1.72×10^{6}	1.23×10^{6}
2H-Pyran-2-one, tetrahydro-	18.927	1316	1.25×10^{5}	1.64×10^{5}	1.67×10^{5}	0.00×00	2.21×10^{5}	3.82×10^{4}	5.55×10^{5}	0.00×00	0.00×00
1-Dodecene	19.383	1336	1.27×10^{5}	1.35×10^{5}	0.00×00	2.48×10^{5}	1.37×10^{5}	2.30×10^{6}	1.58×10^{6}	1.05×10^{6}	5.51×10^{5}
2-n-Heptylfuran	19.712	1351	4.06×10^{4}	9.79×10^{3}	0.00×00	3.66×10^{4}	3.15×10^{4}	9.74×10^{3}	2.05×10^{5}	3.65×10^{4}	4.44×10^{4}
2-Nonenal, (E)-	19.852	1356	9.01×10^{5}	2.20×10^{6}	3.93×10^{6}	8.40×10^{5}	2.05×10^{6}	1.26×10^{6}	1.58×10^{6}	1.66×10^{6}	9.30×10^{5}
2-Decanone	20.222	1373	1.36×10^{5}	6.90×10^{4}	5.47×10^{4}	1.06×10^{5}	9.83×10^{4}	8.86×10^{4}	6.54×10^{5}	1.66×10^{5}	2.11×10^{5}
Decanal	20.395	1373	1.92×10^{6}	1.71×10^{6}	1.53×10^{6}	2.40×10^{6}	1.61×10^{6}	1.73×10^{6}	2.47×10^{6}	3.20×10^{6}	1.79×10^{6}
Octanoic acid	20.563	1388	2.81×10^{6}	2.19×10^{6}	1.70×10^{6}	2.48×10^{6}	2.04×10^{6}	1.67×10^{6}	8.02×10^{6}	5.08×10^{6}	3.75×10^{6}
Benzene, 1,3-bis(1,1-dimethylethyl)-	20.846	1401	1.01×10^{5}	1.82×10^{5}	2.02×10^{5}	1.65×10^{5}	1.40×10^{5}	1.78×10^{5}	9.67×10^{4}	1.81×10^{5}	0.00×00
Benzoic acid	20.942	1406	6.46×10^{5}	5.73×10^{5}	7.14×10^{5}	3.94×10^{5}	9.32×10^{5}	0.00×00	1.11×10^{6}	1.19×10^{6}	1.01×10^{6}
Ethanol, 2-phenoxy-	21.350	1426	8.61×10^{4}	5.75×10^{4}	2.31×10^{4}	1.04×10^{5}	3.37×10^{4}	3.59×10^{4}	2.02×10^{5}	4.66×10^{4}	2.52×10^{4}
2-Decenal, (E)-	21.506	1433	8.11×10^{5}	6.32×10^{5}	6.90×10^{5}	7.74×10^{5}	6.44×10^{5}	5.07×10^{5}	2.45×10^{6}	1.12×10^{6}	8.96×10^{5}
Undecanal	21.897	1452	2.91×10^{5}	0.00×00	0.00×00	2.96×10^{5}	0.00×00	0.00×00	6.05×10^{5}	0.00×00	7.14×10^{4}

Nonanoic acid	21.924	1454	5.79×10^{6}	6.72×10^{6}	4.81×10^{6}	3.83×10^{6}	4.10×10^{6}	3.36×10^{6}	9.60×10^{6}	6.11×10^{6}	4.58×10^{6}
2(3H)-Furanone, 5-butyldihydro-	22.169	1466	5.83×10^{4}	4.31×10^{4}	5.52×10^{4}	5.50×10^{4}	5.79×10^{4}	6.01×10^{4}	2.74×10^{5}	8.74×10^{4}	9.18×10^{4}
2,4-Decadienal	22.342	1474	1.99×10^{5}	4.27×10^{5}	1.36×10^{5}	1.50×10^{5}	9.46×10^{4}	6.97×10^{4}	3.12×10^{5}	2.43×10^{5}	1.06×10^{5}
Butanoic acid, 2,3-dihydroxypropyl ester	22.408	1482	7.08×10^{5}	0.00×00	0.00×00	4.22×10^{5}	0.00×00	0.00×00	8.76×10^{4}	0.00×00	0.00×00
2-Undecenal	22.779	1496	4.68×10^{5}	4.02×10^{5}	4.84×10^{5}	3.33×10^{5}	3.57×10^{5}	2.11×10^{5}	1.56×10^{6}	8.26×10^{5}	6.13×10^{5}
Hexadecanoic acid, methyl ester	22.852	1499	0.00×00	1.10×10^{6}	7.27×10^{5}	1.63×10^{6}	1.96×10^{6}	5.69×10^{5}	5.74×10^{5}	5.60×10^{5}	8.95×10^{5}
Decanoic acid	23.007	1511	1.04×10^{7}	5.50×10^{6}	3.69×10^{6}	8.20×10^{6}	3.37×10^{6}	2.37×10^{6}	6.69×10^{6}	4.90×10^{6}	3.92×10^{6}
Dodecanal	23.078	1515	5.31×10^{5}	7.09×10^{5}	4.69×10^{5}	3.90×10^{5}	6.70×10^{5}	4.35×10^{5}	5.27×10^{5}	5.05×10^{5}	4.01×10^{5}
2(3H)-Furanone, dihydro-5-pentyl-	23.373	1535	4.21×10^{4}	4.44×10^{4}	6.22×10^{4}	4.80×10^{4}	5.40×10^{4}	6.88×10^{4}	2.50×10^{5}	9.23×10^{4}	9.97×10^{4}
1-Dodecanol	23.753	1561	0.00×00	4.37×10^{5}	2.11×10^{5}	3.72×10^{5}	2.29×10^{5}	1.88×10^{5}	2.68×10^{5}	2.28×10^{5}	2.81×10^{5}
Undecanoic acid	23.920	1573	6.16×10^{5}	4.12×10^{5}	2.83×10^{5}	9.79×10^{5}	3.05×10^{5}	2.39×10^{5}	2.86×10^{5}	2.61×10^{5}	2.14×10^{5}
Dodecanoic acid, methyl ester	24.037	1580	5.33×10^{5}	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	0.00×00	1.85×10^{4}
Tridecanal	24.090	1584	1.54×10^{5}	1.84×10^{5}	1.51×10^{5}	1.38×10^{5}	1.92×10^{5}	1.41×10^{5}	2.74×10^{5}	2.06×10^{5}	2.07×10^{5}
2,4-Di-tert-butylphenol	24.448	1611	5.70×10^{5}	5.05×10^{5}	4.83×10^{5}	6.28×10^{5}	5.12×10^{5}	5.10×10^{5}	4.31×10^{5}	4.05×10^{5}	4.83×10^{5}
2H-Pyran-2-one, tetrahydro-6-pentyl-	24.734	1635	3.86×10^{5}	0.00×00	0.00×00	1.03×10^{5}	0.00×00	0.00×00	1.60×10^{4}	0.00×00	0.00×00
Dodecanoic acid	24.949	1651	4.75×10^{6}	6.18×10^{6}	4.62×10^{6}	1.80×10^{7}	5.76×10^{6}	3.62×10^{6}	4.72×10^{6}	4.01×10^{6}	3.45×10^{6}

Diethyl Phthalate	25.491	1696	9.43×10^{5}	5.34×10^{5}	8.66×10^{5}	1.40×10^{6}	4.57×10^{5}	7.00×10^{5}	5.48×10^{5}	6.65×10^{5}	9.29×10^{5}
Dodecyl acrylate	25.909	1725	6.15×10^{5}	9.35×10^{5}	6.50×10^{5}	1.72×10^{6}	6.60×10^{5}	7.41×10^{5}	1.06×10^{6}	6.86×10^{5}	9.45×10^{5}
Tetradecanol	25.909	1725	6.15×10^{5}	9.35×10^{5}	6.50×10^{5}	1.72×10^{6}	6.60×10^{5}	7.41×10^{5}	1.06×10^{6}	6.86×10^{5}	9.45×10^{5}
2-Pentadecanone	26.251	1748	2.76×10^{6}	6.26×10^{5}	1.53×10^{5}	2.82×10^{6}	3.02×10^{5}	9.52×10^{4}	8.32×10^{5}	1.90×10^{5}	1.39×10^{5}
Tetradecanoic acid, methyl ester	26.311	1752	1.26×10^{7}	2.08×10^{6}	1.91×10^{5}	1.36×10^{7}	9.05×10^{5}	8.45×10^{4}	3.12×10^{6}	3.43×10^{5}	5.76×10^{4}
Unknown	26.864	1790	5.83×10^{6}	1.70×10^{6}	4.33×10^{5}	6.55×10^{6}	8.49×10^{5}	2.91×10^{5}	1.88×10^{6}	4.64×10^{5}	2.49×10^{5}
γ-Dodecalactone	27.149	1815	1.06×10^{6}	5.52×10^{5}	1.80×10^{5}	5.39×10^{5}	3.68×10^{5}	1.11×10^{5}	5.34×10^{5}	1.90×10^{5}	9.50×10^{4}
δ-Dodecalactone	27.744	1880	1.88×10^{6}	1.77×10^{6}	8.55×10^{5}	6.88×10^{6}	1.36×10^{6}	6.34×10^{5}	1.39×10^{6}	7.74×10^{5}	5.75×10^{5}
Phytol	27.835	1889	3.26×10^{6}	2.18×10^{6}	1.03×10^{6}	8.32×10^{6}	1.52×10^{6}	7.26×10^{5}	1.81×10^{6}	8.79×10^{5}	5.94×10^{5}
Pentadecanoic acid, methyl ester	27.933	1900	5.77×10^{5}	4.65×10^{5}	2.19×10^{5}	1.60×10^{6}	3.41×10^{5}	1.52×10^{5}	3.89×10^{5}	1.98×10^{5}	1.15×10^{5}

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REVIEW ARTICLE

Volatile organic compounds in beef and pork by gas chromatography-mass spectrometry: A review

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The aroma of meat is an integral part of flavor and therefore an essential element for consumer acceptance. Aroma compounds are challenging to extract, concentrate, separate, identify, and quantify due to the complexity of meat. A wide range of volatile compounds of different properties (molecular weight, polarity, vapor pressure) are present in raw meat at varying concentrations, and further exacerbated post cooking due to the creation of additional volatiles through mainly Maillard reactions and lipid oxidation reactions. The volatiles identified in cooked meats include hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, lactones, ethers, heterocyclic constituents, and sulfur-/halogen-containing components. From published research, some of the key volatiles influencing cooked beef/pork flavor include hexanal, octanal, nonanal, 1-nonanal, (E,E)-2,4-decadienal, methional, 3-methyl-1-butanol, methanethiol, 2-furfurylthiol, 2-metyl-3-furanthiol, 3-mercapto-2-pentanone, 4-hydroxy-2,5-dimethyl-3-(2H)-furanone, 1-penten-3-one, 2-pentyl-furan, *N*-morpholinomethyl-isopropyl=sulfide, and methyl butyrate. GC-MS is the method of choice for their separation and identification. The technical options available have never been greater to elucidate these key volatiles impacting sensory perception. This review presents a summary of the main extraction/concentration techniques, highlighting their potential pros and cons, how advances in two-dimensional gas chromatography may help elucidate more key aroma compounds, and outlines the benefits of olfactometry to determine the main odor-active volatiles in beef and pork.

KEYWORDS
gas chromatography-mass spectrometry, headspace, purge-and-trap, thermal desorption, volatile organic compounds

Abbreviations: CAR-PDMS, carboxen–PDMS; CW DVB, carbowax–DVB; CW-TPR, CW–template resin; DE, distillation extraction; DHS, dynamic headspace; DI-SPME, direct immersion; ECD, electron capture detectors; FID, flame ionization detectors; GC-MS, gas chromatography-mass spectrometry; GC-O, gas chromatography-olfactometry; GCxGC, two-dimensional gas chromatography; Hi-Sorb, high capacity sorptive extraction; HS-SPME, headspace solid phase microextraction; LLE, liquid–liquid extraction; P&T, purge-and-trap; PA, polyacrylate; PDMS, polydimethylsiloxane; PDMS-DVB, PDMS–divinylbenzene; PEG, carbowax-polyethylene glycol; Q, single quadrupole; SAFE, solvent assisted flavor evaporation; SBSE, stir bar sorptive extraction; SDE, simultaneous distillation extraction; SHS, static headspace; SPDE, solid-phase dynamic extraction; SVOC, semi-VOC; TD, thermal desorption; TID, thermal ionization detectors; TOF, time-of-flight; TQ, triple quadrupole; VOC, volatile organic compounds.

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1 | INTRODUCTION

Meat is a complex, heterogeneous mixture composed of a multitude of different volatile compounds that contribute to its overall sensory perception [1]. Flavor, conformed by taste and aroma, is one of the main factors that instigates consumer acceptance of foods [2]. Flavor is the result of the combination of taste compounds registered on the tongue and volatile compounds moved collectively throughout the mouth, sinus, and nasal cavities (orthonasal- and retronasal-olfaction) to the odor epithelium in the nose [3]. Although the basis for meat flavor, such as beef and pork, is established in the primary production phase through choice of breed and feed, it is also influenced by the slaughtering process, and the aging and cooking processes of the meat [3].

Beef and pork flavor typically comprises of hundreds of volatile organic compounds (VOC), with trace-level analytes often having the greatest effect on perceived aroma because the odor activity of the individual VOC can be more important than their concentration [4]. The analysis of VOC firstly requires their extraction and concentration [1], followed by separation typically undertaken using gas chromatography (GC), and finally identification and possible quantification using one or more detection systems. Due to the wide variety of potential VOC, differing in polarity, volatility, vapor pressure and molecular weight, a wide range of different extraction techniques exist. It is also beneficial to use the human nose as a detector due to its sensitivity, but also as panelist(s) can provide specific aromatic descriptors for volatiles, and thus potentially confer additional information in relation to its perceived intensity, and thus identify those likely contributing most to consumer perception. Volatile concentration is required for olfactometry analysis as extraction and separation alone dilute the odor profile making it very difficult, if not impossible to discern individual VOC (although this can be product specific). Previous studies have identified that the real volatile fraction that reaches the human receptors when a sample is sniffed is better represented by volatiles obtained by dynamic headspace (DHS), purge-and-trap (P&T), headspace solid phase microextraction (HS-SPME) techniques [5], or thermal desorption (TD). However, no single extraction method or approach is likely to provide a complete volatile profile of a sample, due to inherent bias associated with the extraction and separation techniques of choice (differences in chemistry of the phases, capacity, etc.).

The analysis technique should be selected based on whether the aim is to target some specific volatiles as a targeted approach, or as an untargeted approach in an attempt to obtain as much of the representative volatile profile as possible. The following factors ought to be also taken into consideration: (a) the sample; the composition of the sample matrix (particular attention to the moisture content and pH), the presence of interfering compounds (especially fat and protein), the amount of sample that requires analysis, the homogeneity of the sample, and its availability (all of which should influence the amount of sample required), (b) the analytes of interest; the potential concentration and range present, their thermal stability, the potential range of their boiling points, and volatility, and (c) the extraction methods and equipment availability, cost, time, accuracy, precision, and reproducibility [6].

The aim of this review is to provide an overview of the sample preparation techniques used to access VOC in beef and pork by GC-mass spectrometry (MS) and GC-olfactometry (GC-O), to highlight potential advantages and disadvantages of specific extraction techniques, and to describe the main volatiles contributing to the characteristic flavor of beef and pork to date.

A data search using the major databases (PubMed, ScienceDirect, SpringerLink, ResearchGate) with the keywords “dynamic headspace,” “static headspace,” “purge-and-trap;” “thermal desorption,” “volatile organic compounds,” “gas chromatography;” “mass spectrometry,” “meat,” “beef,” “pork,” “flavor” was carried out to identify the relevant original articles for this review. The research highlighted that there is a disparity of information on sample preparation techniques for VOC analysis by GC-MS and therefore this timely review provides a relevant up to date summary of the four most widely used extraction techniques for meat products, DHS, static headspace (SHS), HS-SPME, and TD; and includes novel analytical approaches such as two-dimensional gas chromatography (GCxGC) which are becoming more widespread due to advances in non-cryogenic flow control and software.

2 | DEVELOPMENT OF MEAT FLAVOR

In summary, meat flavor development is primarily dependent on two factors: reactant mixture or intrinsic factors (i.e., chemical composition and pH) and reaction conditions or extrinsic factors (i.e., thermal kinetics as a result of varied time and temperature exposure). Flavor development, therefore, is highly dependent on the reactant mixture (precursor compounds of flavor) and reaction conditions (cooking), and is described as a complex system of products and interactions. Many VOC are the final products of cooked meat flavor development and therefore their detection presents an opportunity to better understand meat flavor development and how chemical flavor components relate to perceived flavor.

The final composition of VOC in beef and pork products is determined by breed, gender, diet and age of an animal; slaughter process; duration and condition of meat storage; type of muscle; preparation of meat, and type of additives applied as well as condition of heat treatment (cooking, roasting, smoking) and, any technological processes undertaken [7]. Among the different factors which influence the meat flavor, animal diet is the most important, especially its lipid content, as it is the main source of aromatic volatile compounds [8]. The flavor of beef and pork is complex as it depends on the interaction of volatile (aroma) and non-volatile (taste) compounds [9]. Raw meat, in spite of its weak aroma and blood-like taste, constitutes a rich matrix of non-volatile precursors (amino acids, peptides, reducing sugars, vitamins, nucleotides, and fatty acids) and a diverse pool of VOC, some of which are responsible for its flavor [3]. Beef and pork develop its aroma characteristics during cooking as a result of interaction of precursors derived from lean and fat components [10]. This occurs via four pathways including (a) Maillard reaction of amino acids or peptides with reducing sugars, (b) lipid oxidation, (c) interaction between Maillard reaction products with lipid-oxidized products and (d) thiamine (vitamin B1) degradation. Maillard reaction products come from high temperature rapid cooking whereas lipid products are generated from low temperature slow cooking [11]. Even though many different VOC from a range of chemical classes are present, it is estimated that only a small portion (<3%) are capable of imparting odors to the food [12], as they need to be at a concentration above their odor thresholds to be perceived. As the odor thresholds of VOC vary markedly and are impacted mainly by product composition, a less abundant VOC can have a greater aromatic impact than others present at significantly higher concentrations.

2.1 | Maillard reaction

The Maillard reaction is a redox reaction between the amine group of an amino acid and the carbonyl group of a reducing sugar. A cascade of reactions, including (1) sugar-amine condensation, the Amadori rearrangement, (2) sugar breakdown and dehydration, Strecker degradation, and (3) aldol condensation, aldehyde-amine condensation and formation of heterocyclic nitrogen compounds via Schiff bases pathways, create a number of VOC that produce desirable flavors in cooked meats [13, 14] (Figure 1). If the initial reducing sugar is an aldose monosaccharide that has an aldehyde group (ribose or glucose), it forms an aldosylamine-N-substituted, which is rearranged to form an intermediary Amadori. If the reducing sugar is a ketose monosaccharide that has a ketone group (ribulose or fructose), Heyns rearrangement of ketosylamine-N-substituted and the corresponding Heyns intermediary are produced [15]. Next, the Amadori/Heyns products are degraded, which leads to dehydrations and deaminations that produce carbonyl compounds such as deoxysones and deoxyreductones [15]. Then, primary compounds such as furfural, furanone derivatives, hydroxyketones, and dicarbonyl compounds are created. More complex products derived from other amines, amino acids, aldehydes, hydrogen sulfide, and ammonia are also formed. The final stage of the Maillard reaction, the Strecker degradation is the most important step for flavor formation, as it provides routes by which nitrogen and sulfur can be introduced into heterocyclic compounds [16]. Strecker degradation is initiated by the reaction between carbonyl compounds and an amino acid forming two important intermediate compounds, α-aminoketones or aminoalcohols and an aldehyde. The Strecker aldehyde itself contributes to the flavor of the meat [17] and the α-aminoketones are key precursors for heterocyclic compounds, such as pyrazines, pyrroles, oxazoles, thiophenes and thiazoles, and furans, all of which are also important classes of aroma compounds in meat [11]. The amino acid cysteine has long been associated with food flavor since its decomposition via Strecker degradation leads to numerous low aroma threshold sulfur VOC (hydrogen sulfide, ammonia and acetaldehyde), whereas methionine will yield methional, 2-propenal, methanethiol and dimethyl disulfide [15, 17]. Hydrogen sulfide per se and its reaction products with aldehydes, furfural and furanones have also been proven to be a diverse source of flavor compounds in meat products [18]. Some of these compounds, together with carbonyl compounds produced in the Maillard reaction, provide intermediates for reactions giving rise to important aroma compounds [17]. The reaction also leads to the formation of melanoidins, brown and high molecular weight polymers, from the condensation of cyclic compounds [13]. The myriad of compounds derived from the Maillard reaction are responsible for the flavors generally described as roasted, browned, meaty, and caramelized [11] and are key aromatic volatiles influencing sensory perception.

2.2 | Lipid oxidation

Lipid oxidation is a major cause of meat quality deterioration during storage, but low levels of lipid oxidation can enhance cooked meat flavor [17]. Fatty acids and lipids contribute greatly to the generation of aromatic VOC that are characteristic of cooked meat [17]. Therefore, both fat content and the fatty acid profile are critical to the formation of many volatiles in cooked meat [19]. These are influenced by diet, which highlights the potential of diet

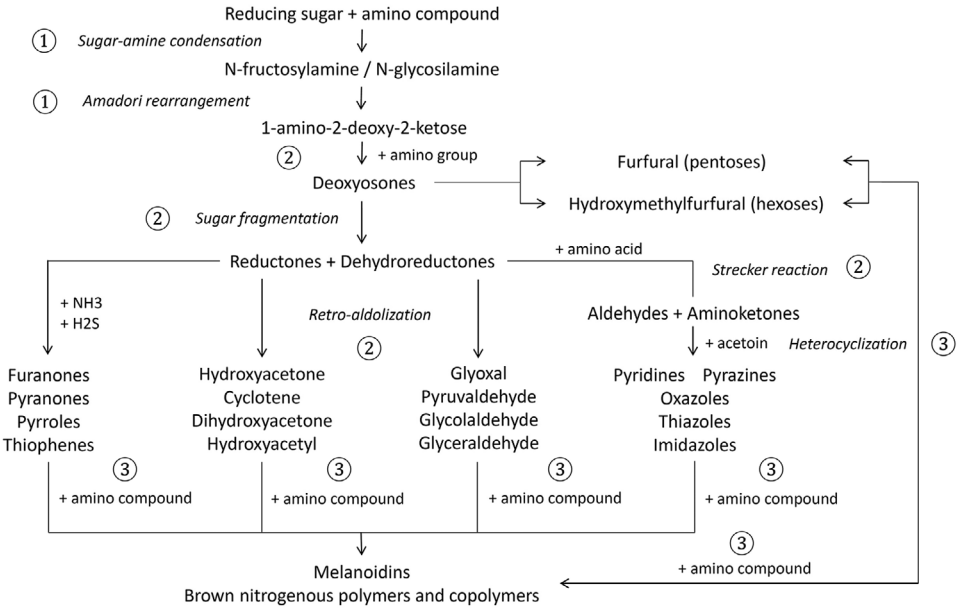


FIGURE 1 Schematic overview of the Maillard reaction showing the main-end products contributing to flavor (adapted from Sun et al. [16])

in relation to their creation and the sensory perception of cooked meats. Zhao et al. [20] suggested that the fatty acid composition is unique to each species, therefore species flavor must be associated with lipid degradation during cooking [7]. Arshad et al. [21] also postulated that the lean tissue is responsible for the "meaty" flavor found in all species. However, the phospholipids in the cell membrane of the lean tissue, even though a small fraction of the total lipid content in beef, are extremely important to the formation of volatile flavor components [22]. Intramuscular fat and adipose tissues are now thought to be the main source of volatile flavor components in meat. This fat and adipose tissue contain different proportions of saturated and unsaturated fatty acids, which when degraded and oxidized provide a prolific number of VOC [19]. On the contrary, high levels of polyunsaturated fatty acid can cause undesirable flavors as they are more susceptible to oxidation and also inhibit the production of some Maillard reaction products [19]. Examples of VOC derived from lipid degradation in cooked meat include aldehydes, alcohols, ketones, esters, carboxylic acids, and aliphatic hydrocarbons [17], all of which can give rise to "fatty" aromas in cooked meat [10].

2.3 | Interactions between Maillard reaction products and lipid-oxidized products

Maillard reaction and lipid oxidation should be considered simultaneously to fully understand the reactions involved and the products formed in complex matrices involving lipids, amino acids and carbohydrates. Maillard reaction products are capable of promoting and reducing lipid oxidation. For example, Amadori products have been shown to increase phospholipid oxidation, and non-enzymatically browned proteins can reduce lipid oxidation. On the other hand, lipid oxidation products can modify the Maillard reaction by promoting or preventing the reaction, or by reacting with some intermediates and producing compounds different from those commonly formed in the absence of lipids. One example of this is the development of aroma in cooked meat, where the presence of lipids is essential for the development of pleasant flavors [23]. VOC that can be considered as products of Maillard/lipid interactions have been identified in studies with model systems [24, 25]. The majority of these are heterocyclic compounds containing one or more atoms of nitrogen or sulfur and are characterized by the presence of a long-chain alkyl substituent with four or more carbon atoms [8]. Some of the compounds that are either derived or could be derived from interactions of Maillard reactions and lipids include alkanethiols (1-heptanethiol, 1-octanethiol), thiophenes (2-butyl/pentyl/hexyl/heptylthiopene), pyridines (2-pentylpyridine), and some alkylsulfides, alkylamines, pyrroles, and thiazoles [26].

2.4 | Thiamin degradation

Thiamin degradation is an important flavor generation pathway in cooked meat. Thiamin is susceptible to ther-

mal degradation and produces a multitude of flavor compounds [15], most of them contain one or more sulfur and/or nitrogen atoms [10]. The primary products of thermally-degraded thiamin include 4-methyl-5-(2-hydroxyethyl)thiazole, which subsequently degrades to form other thiazoles, and 5-hydroxy-3-mercaptopentan-2-one. These can be further degraded to other sulfur-containing compounds, such as thiophenes and specific furans [27]. Some significant thiamin thermal degradation products are 2-methyl-3-furanthiol, bis(2-methyl-3-furyl) disulfide, 3-thiophenethiol, 2-formyl-5-methylthiophene and 2-methyl-3-(methyldithio) furan, all of which produce intense meaty aromas [28]. Some of these VOC derived from thiamin degradation can also be produced from the Maillard reaction between cysteine and ribose, the Strecker reactions of sulfur amino acids and their interactions [15].

3 | SPECIFICITY OF BEEF AND PORK FLAVOR COMPOUNDS

Different classes of VOC contribute their own attributes to the overall meat flavor. The volatiles identified in cooked beef and pork in general include most types of organic compounds: hydrocarbons, alcohols, aldehydes, ketones, carboxylic acids, esters, lactones, ethers, heterocyclic constituents (furans, pyridines, pyrroles, oxazoles and oxazolines, thiazoles and thiazolines, and thiophenes), and other sulfur- as well as halogen-containing components. The odor threshold is a very important concept to understand in discussing the potential sensory importance of any VOC. The odor threshold is the minimum concentration (usually provided in water, a solvent, fat, or air) at which the volatile compound can be perceived orthonasally or retronasally, and is typically expressed in either parts per million (ppm) or parts per billion (ppb) [29]. Nitrogen- and sulfur-containing heterocyclic compounds that are derived from Maillard reactions tend to have the lowest odor thresholds. Lipid-derived VOC, such as ketones and alcohols, tend to have higher odor thresholds and therefore a greater concentration is required before these can be perceived [30]. The most odor-active compound found in any product to date is 2-methylfuran-3-thiol (meaty aroma) with an odor threshold of 0.00003 μg/kg [31, 32].

To date a large number of VOC contributing to the flavor characteristics of cooked beef have been identified [10]. Some of these key flavor compounds include methional (cooked potato aroma), E-2-undecenal (sweet, fruity, fatty aroma), E-2-dodecenal (sweet, fruity, roasty aroma), decanal (sweet fruity, meaty aroma), heptanal (fruity, fatty, green aroma), and 2-methylbutanal (pungent, sweet, roasty aroma) [33]. Others such as dimethyl sulfide (sweet, fruity, gas aroma), 2-butanone (chemical, burnt, gas aroma), ethyl acetate (caramel, sweet aroma), 3-methylbutanal (meaty, rotten, fatty, aldehyde, pungent, green, apple, malt aroma), 2-heptanone (citrus, grapefruit, floral, fruity, spicy, cinnamon aroma), nonanal (sweet, green, citrus, floral, waxy aroma), and 2,4-nonadienal (fatty, meaty, burnt, chocolate) are key odors in Angus beef [34, 35]; 2-furfurylthiol (roasty aroma), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (roasted almonds, sweet aroma), and 2-methyl-3-furanthiol (meaty, sweet, sulfurous, roast/boiled meat aroma) are important in boiled beef [36]; whilst 2-ethyl-3,5-dimethyl pyrazine (burnt, fragrant, meaty, green aroma) and 2,3-diethyl-5-methylpyrazine (meaty, roasty, fragrant, sweet aroma) are key VOC in roast beef [37]. Some of the individual volatile compounds that have been found to be characteristic of the distinct aroma flavors of beef are listed in Table 1. As previously mentioned, lipid-derived VOC are important, the majority of which include saturated and unsaturated aldehydes with six to ten carbons. The most common VOC found in cooked beef are hexanal (green, fatty, grassy, sweet, fresh notes) and nonanal (sweet, fatty, green, pungent, sea, citrus, citronella grass, floral, grassy, waxy notes) which are key products of enzymatic oxidation of unsaturated fatty acids. This is explained by the fact that the primary substrate for these aldehydes, oleic acid (omega-9 monounsaturated fatty acid), is the most abundant fatty acid and omega-6 fatty acid in beef [11].

The VOC in cooked pork have also been documented [28]. Analysis of VOC in pork meat has revealed nine chemical classes including aldehydes, alcohols, ketones, furans, sulfides, esters, acids, phenols, and hydrocarbons, as well as small amounts of other VOC such as alkenes and pyrroles [38, 39]. The VOC thought to contribute to the flavor of pork are hexanal (grass), octanal (fresh), 1-nonanal (delicate), (E,E)-2,4-decadienal (broth), 3-methyl-1-butanol (pungent), 1-penten-3-one (onion), 2-pentyl-furan (ham), N-morpholinomethyl-isopropyl-sulfide (meaty) and methyl butyrate (apple-like) [38]. Seven other odor-active compounds (hexanal (green grass), heptanal (fatty, putty), nonanal (fatty, floral, wax), 1-octen-3-ol (mushroom), 2-pentylfuran (pungent, sweet), 2-ethylfuran (rubber, pungent), and dimethyl disulfide (cooked cabbage)) have also been identified as the main contributors to the integral flavor of boiled pork due to their higher odor activity values [40].

4 | SAMPLING/EXTRACTION METHODS

The most important step prior to GC determination of an analyte is sample preparation; it is a key step in relation to VOC analysis in meat. Sample preparation involves

TABLE 1 Principal flavor VOC most frequently detected in beef, their odor description, extraction technique, and polarity of GC column used for analysis

Compound	CAS no.	Odor description	Extraction technique	Polarity column	Ref.
Aldehydes[a]					
Propanal	123-38-6	Caramel, sweet, alcoholic, "cooked," broth, spicy	HS/SPME	P/NP	[84, 151]
Butanal	123-72-8	Smoky, fish, amylic, aldehyde	HS/SPME	P/NP	[84, 151]
Pentanal	110-62-3	Almond, malt, pungent, acrid	NR/SPME	NR/NP	[84, 152]
Hexanal	66-25-1	Green, fatty, grassy, sweet, fresh	HS/SPME/TD	P/NP	[33, 84, 98, 151, 152]
Heptanal	111-71-7	Fruity, fatty, sweet, oil, green, rancid	HS/SPME/TD	P/NP	[33, 84, 98, 151, 152]
Octanal	124-13-0	Green, lemon, citrus, aldehyde, fatty, orange peel, soapy, honey	HS/SPME/TD	P/NP	[84, 98, 151, 152]
Nonanal	124-19-6	Sweet, fatty, green, pungent, sea, citrus, citronella grass, floral, grassy, waxy	HS/SPME/TD	P/NP	[33, 84, 98, 151, 152]
Decanal	112-31-2	Sweet, fruity, aldehyde, roasty, fatty, rancid, meaty, burnt, overcooked	HS/SPME/TD	P/NP	[33, 84, 98, 151, 152]
Undecanal	112-44-7	Sweet, pungent, green, alcoholic, floral, lemon, sea	HS	P/NP	[33, 151]
Furfural	98-01-1	NR	TD	NP	[98]
Benzaldehyde	100-52-7	Almond oil, bitter almond, burning aromatic taste	NR/SPME/TD	NR/NP	[84, 98, 152]
Phenylacetaldehyde	122-78-1	Sweet, fruity, flowery, honey	HS/SPME	NP	[33, 84, 156]
Methional	3268-49-3	Cooked potato, meat broth	HS/TD	NP	[33, 98, 153]
2-methylbutanal	96-17-3	Pungent, sweet, roasty	HS/SPME/TD	NP	[33, 84, 98]
2-nonenal	2463-53-8	Cardboardy, orris, fat, cucumber, paper	NR	NR	[152]
2-tridecenal	7069-41-2	Sweet, strong, spicy	NR	NR	[152]
2,4-decadienal	2363-88-4	Deep fat, citrus, orange, grapefruit	NR	NR	[152]
2,4-nonadienal	6750-03-4	Fat, wax, green, watermelon, geranium, pungent	NR	NR	[152]
3-methylbutanal	590-86-3	Meaty, fish, rotten, aldehyde, fatty, solvent-like, pungent, green, apple, malt	HS/SPME/TD	P/NP	[33, 84, 98, 151, 152]
E-2-hexenal	6728-26-3	Green	NR/SPME	NR/NP	[10, 84]
E-2-heptenal	18829-55-5	Fatty	NR/SPME	NR/NP	[10, 84]
E-2-octenal	2548-87-0	Aldehyde, green, floral, dienal, fatty, cardboard, amine, nut	HS/SPME	P/NP	[84, 151–153]
E-2-nonenal	18829-56-6	Fatty, pungent, fragrant, fruity, roasty, wood, nutty, dienal	HS/SPME	P/NP	[33, 84, 151, 153]
E-2-decenal	3913-81-3	Pungent, green, sweet, fruity, fatty, tallow, orange	HS	P/NP	[33, 151, 152]
E-2-undecenal	53448-07-0	Sweet, fruity, fatty	HS	NP	[33]
E-2-dodecenal	20407-84-5	Sweet, fruity, roasty, pungent	HS	NP	[33]
E,E,2,4-heptadienal	4313-03-5	Aldehyde, green, broth, spicy, nut, fat	HS	P	[151, 152]

(Continues)

TABLE 1 (Continued)

Compound	CAS no.	Odor description	Extraction technique	Polarity column	Ref.
E,E,2,4-octadienal	30361-28-5	Mild, alcoholic, floral, green, lemon, aldehyde, sea	HS	P	[151]
E,E,2,4-nonadienal	5910-87-2	Fatty, dienal, aldehyde, insect, fatty, bad, rancid	HS/SPME	P/NP	[84, 151, 153]
E,E,2,4-decadienal	25152-84-5	Sweet, rubbery, fatty, pungent, plastic	HS/SPME	P/NP	[33, 84, 151, 153]
Ketones[b]					
1-octen-3-one	4312-99-6	Fresh, mushrooms, pungent, rubbery, old water, cellar	HS/SPME	P/NP	[33, 84, 151, 153]
1-nonen-3-one		Mushroom	HS	P	[151]
2-butanone	78-93-3	Chemical, burnt, gas, chocolate	HS	NP	[34]
2-heptanone	110-43-0	Citrus, grapefruit, limonene, floral, cheese, fruity, spicy, cinnamon	HS/SPME	P/NP	[84, 151, 152]
2-octanone	111-13-7	Fruity, musty	HS/SPME	NP	[84, 153]
2-nonanone	821-55-6	Hot milk, soap, green, fruity, floral	NR	NR	[152]
2-decanone	693-54-9	Fruity, musty	HS	NP	[153]
2-undecanone	112-12-9	Fruity	NR	NR	[10]
2-dodecanone	6175-49-1	Fruity, musty	HS	NP	[153]
2-methyl 3-octanone	923-28-4	Herb, butter, resin, gasoline	NR	NR	[152]
2,3-butanedione	431-03-8	Sweet, buttery	HS	P/NP	[33, 151]
2,3-pentanedione	600-14-6	Buttery, lemon-like, sweet, fruity	HS	NP	[33]
2,5-dimethyl-4-hydroxy-3(2H)-furanone	3658-77-3	Roasted almonds, sweet	HS	NP	[33]
3-octanone	106-68-3	Fruity, nutty, moldy, fatty, earthy	HS	NP	[33]
3-octen-2-one	1669-44-9	Nut, earthy, spicy, sweet, mushroom	NR	NR	[10]
3-[(2-furanylmethyl)dithio]-2-butanone	61295-44-1	Onion, burnt rubber, burnt wood	HS	NP	[154]
3-[(2-furanylmethyl)dithio]-2-pentanone	180031-78-1	Sweet, onion, roast nuts	HS	NP	[154]
4-methyl-3-penten-2-one	141-79-7	Sweet, chemical	HS	NP	[34]
6-methyl 2-heptanone	928-68-7	Cloves, menthol	NR	NR	[152]
Dihydro-5-propyl-2(3H)-furanone	105-21-5	Fruity, fatty, sweet, pungent, roasty	HS	NP	[33]
Alcohols[c]					
Ethanol	64-17-5	Grilled, acetaldehyde-like			[10]
Propanol	71-23-8	Alcoholic	NR	NR	[152]
1-pentanol	71-41-0	Fuel oil, fruit, balsamic	NR/SPME	NR/NP	[84, 152]
1-hexanol	111-27-3	Woody, cut grass, chemical-winey, fatty, fruity	NR	NR	[152]

(Continues)

TABLE 1 (Continued)

Compound	CAS no.	Odor description	Extraction technique	Polarity column	Ref.
1-heptanol	111-70-6	Fragrant, woody, oily, green, fatty, winey, herb	NR	NR	[152]
1-octanol	111-87-5	Penetrating aromatic odor, fatty, waxy, citrus, oily, walnut, moss, chemical, metal, burnt	NR/SPME	NR/NP	[84, 152]
1-octen-3-ol	3391-86-4	Mushroom	NR/SPME	NR/NP	[84, 152]
2-ethyl-1-hexanol	104-76-7	Resin, flower, green	NR	NR	[152]
2-hexen-1-ol	928-95-0	Green, sharp, leafy, fruity, unripe banana	NR	NR	[152]
2-octen-1-ol	22104-78-5	Green, citrus	NR/SPME	NR/NP	[84, 152]
Pyrazines[d]					
Pyrazine	290-37-9	Nutty, cracker, roasted	SPME	NP	[84]
2-ethyl-5-methylpyrazine	13360-64-0	Fruity, sweet, pungent	HS/TD	NP	[33, 98]
2-ethyl-3,5-dimethylpyrazine	13925-07-0	Burnt, fragrant, meaty, green	HS/TD	NP	[33, 98, 158]
2-ethyl-3,6-dimethylpyrazine	13360-65-1	Burnt, roasty, pungent, earthy	HS/SPME	NP	[33, 84]
2-ethenyl-5(6)-methylpyrazine	13925-08-1/13925-09-2	Roasty break-like, cooked rice, coffee-like, popcorn, smoky	HS	NP	[33]
2-ethenyl-3,6(5)-dimethylpyrazine	80935-98-4	Sweet, cooked rice, fatty, pungent	HS	NP	[33]
2-isopentyl-3,6-dimethylpyrazine	18433-98-2	Sweet, fragrant, fatty, fruity, pungent	HS	NP	[33]
2,3-diethyl-5-methylpyrazine	18138-04-0	Meaty, roasty, fragrant, sweet	HS/TD	NP	[33, 98, 155]
2,5-dimethylpyrazine	123-32-0	Fried rice, popcorn, pungent, green, roasted, earthy, nutty	NR/SPME	NR/NP	[10, 84]
3-ethyl-pyrazine	13925-00-3	NR	TD	NP	[98]
3-ethyl-2,5-dimethylpyrazine	13360-65-1	NR	TD	NP	[98]
3,5-diethyl-2-methyl-pyrazine	18138-05-1	NR	TD	NP	[98]
Sulfur & nitrogen containing compounds[e]					
Benzothiazole	95-16-9	Stewed, gravy, gas, roasted	HS	NP	[34]
Bis(2-methyl-3-furyl)disulfide	28588-75-2	Meaty, boiled meat	HS	NP	[153, 154]
Bis(2-furanylmethyl) disulfide	4437-20-1	Roast, brazil nuts, baked mushroom	HS	NP	[154]
Dimethylsulfide	75-18-3	Sweet, gas, fruity	HS	NP	[34]
Dimethyl disulfide	624-92-0	Moldy, pungent, rubbery, onion-like	HS/SPME	NP	[33, 84]
Dimethyl trisulfide	3658-80-8	Gas, burnt, gravy, fragrant, musty, roasty, rubbery	HS/SPME	NP	[33, 34, 84]
Methanethiol	74-93-1	Sweet, gas	HS	NP	[34]
2-acetylthiazole	24295-03-2	Roasted	HS	NP	[34, 153]
2-acethylthiophene	88-15-3	Sulfurous, sweet	NR	NR	[10]

(Continues)

TABLE 1 (Continued)

Compound	CAS no.	Odor description	Extraction technique	Polarity column	Ref.
2-formyl-5-methylthiophene	13679-70-4	Sulfurous	NR	NR	[10]
2-furfurylthiol	98-02-2	Roasty	NR	NR	[10]
2-mercapto-3-pentanone	17042-24-9	Brothy, mashed potatoes meaty, roast meat	HS	NP	[154]
2-methyl-3-furanthiol	28588-74-1	Meaty, sweet, sulfurous, roast meat, boiled meat	HS	NP	[34, 154]
2,4-dimethylthiazole	541-58-2	Rubbery, moldy, fruity, pungent	HS	NP	[33]
3-mercapto-2-butanone	40789-98-8	Fried onion, sulfury, cooked meat	HS	NP	[154]
4,5-dimethylthiazole	3581-91-7	Smoky, roasty, fragrant, nutty	HS	NP	[33]
Other					
Ethyl acetate	141-78-6	Caramel, sweet	HS	NP	[34]
Butyric acid	107-92-6	Sweet, unpleasant	HS	NP	[33]
Hexanoic acid	142-62-1	Goaty	NR	NR	[152]
Nonanoic acid	112-05-0	NR	TD	NP	[98]
Decanoic acid	334-48-5	NR	TD	NP	[98]
1,3-Bis(1,1-dimethylethyl)benzene	1014-60-4	Cooked beef	NR	NR	[152]
2-methyl-3-(methyldithio)furan	65505-17-1	Meaty, sulfury, fatty	HS	NP	[154]
2-[(methyldithio)methyl]fu	57500-00-2	Brothy, spices, roast, fatty	HS	NP	[154]
2-pentylfuran	3777-69-3	Green bean, butter	NR/SPME	NR/NP	[84, 152]

[a]Approximate odor threshold [a,e] ppb → ppm; [b] ppm [d] ppb.
HS, headspace; NP, non-polar; NR, not reported; P, polar; SPME, solid-phase microextraction; TD, thermal desorption.

converting a non-homogenous mixture (meat), into a suitable sample for analysis. Sample preparation procedures for VOC analysis usually involve extraction, concentration, possibly clean-up, and sometimes derivatization to convert non-volatile components to volatile components to transfer to vapor phase prior to injection onto the GC [41]. The basic purpose of all extraction methods is to purify (as much as possible) and to concentrate analytes selectively in one or multiple phases. Many different VOC extraction/concentration techniques exist which vary considerably and the choice has a significant impact on the selectivity and sensitivity of an analytical method [42] (Table 2). It is worth reiterating that no single VOC extraction method can provide a complete representative volatile profile as each has inherent degrees of bias toward specific VOC dependent upon molecular size, polarity, and vapor pressure. Other factors concerning potential advantages or disadvantages for each technique relate to the degree of automation/length of time, cost, sensitivity, practicality, risk assessment, reproducibility, potential for artifact formation and ease of use.

For the purpose of this review some of the most common extraction techniques have been summarized into 3 groups (Figure 2):

4.1 | Distillation/solvent extraction techniques

Traditionally distillation/solvent techniques were favored for volatile extraction in foods, but subsequently as throughput, reproducibility and green chemistry have become more important that this has seen a movement toward more automated and flexible multipurpose extraction/enrichment techniques.

4.1.1 | Distillation extraction

Distillation is a physical separation based on the vaporization of the different components of the mixture to be separated. Typically, a mixture is heated, VOC are

TABLE 2 Comparison of headspace, purge-and-trap, HS-SPME and TD GC techniques

	Matrix	Sensitivity	Range of compounds analyzed				Sample weight (g)	Sample procedure	Sample prep. (min)	Sample automate
			Gas	Volatile	Semi-volatile	Non-volatile				
Headspace	L, S	ppm	✓	✓	✗	✗	0.1–10	E, C, CU, D	5–10	Yes
Purge-and-trap	G, L, S	ppb	✗	✓	✓	✗	5–1000	E, C, CU	10–30	No
HS-SPME	G, L, S	ppt	✗	✓	✓	✗	0.1–10	E, C, CU, D	5–15	Yes
TD	S	ppb	✓	✓	✓	✗	0.1–1	E, C, CU, D	1–2	No

C, concentration; CU, clean-up; D, derivatization; E, extraction; G, gas; HS-SPME, headspace solid-phase microextraction; L, liquid; ppb, parts per billion (10^{-9}); ppm, parts per million (10^{-6}); ppt, parts per trillion (10^{-12}); S, solid; TD, thermal desorption.
Adapted from Manura et al. [6].

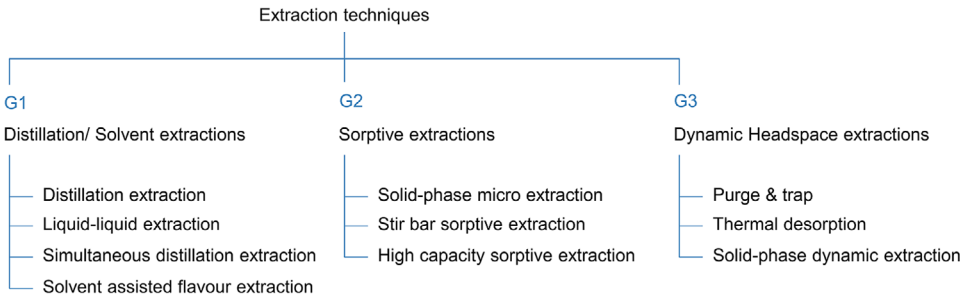


FIGURE 2 Common extraction techniques for VOC in meat

enhanced in the headspace, separated, and then condensed back into a liquid. As a result, each component can be recuperated separately in different fractions. The main advantage of all distillation techniques is obtaining a relatively clean liquid extract from the sample. One of the main disadvantages of distillation with a carrier liquid lies in its destructiveness, the need for large volumes of solvents nor does it provide a complete extract as the total amount of a given component is distributed between its liquid and vapor phases [43]. Thus, its more suited for the extraction of semi-volatile and heat stable VOC and less so for more volatile VOC. Distillation is one of the oldest methods used mainly for the extraction of oils from plants [44].

4.1.2 | Liquid–liquid extraction

Liquid–liquid extraction (LLE), also known as partitioning, is one of the oldest extraction techniques. The principle of LLE involves transferring an analyte from an aqueous matrix into an extraction solvent, usually water and a nonpolar organic solvent. The extraction solvent used for LLE should be immiscible in the aqueous matrix so that the two liquids can be easily separated. LLE process offers several advantages such as provides high capacity of the extractant with a high selectivity of separation and is relatively simple to perform. Disadvantages of LLE include use of large solvent volumes, long extraction times, and multiple extractions may be required in order to remove the majority of the analyte from the matrix [45]. LLE is common in the chemical and pharmaceutical industries and in biotechnology, but much less so in food processing. Its applications to food are generally restricted to isolated cases, such as edible oils [46].

4.1.3 | Simultaneous distillation extraction

The simultaneous distillation extraction (SDE) technique combines steam distillation together with solvent extraction. Briefly, the sample is initially prepared in an aqueous solution. An immiscible organic solvent is used as the extractant. Both solutions are heated separately in glass flasks connected to ports of a glass manifold. The sample and extractant vapors are transferred to the central part of the manifold, where they are condensed. The extraction is initiated as the gaseous and liquefied analytes, sample solvent, and extractant come into contact. The sample solvent and the extract undergo phase separation in a U-shaped glass tube. The two liquids are directed to the sample and extractant flasks, respectively [47]. Despite its general practicality, SDE has several limitations, which hinder its widespread use: it requires practical expertise (a high degree of temperature control, solvent should not mix with

the sample matrix) and extraction times are long. However, the SDE technique is particularly suitable for the extraction of VOC present in complex matrices.

4.1.4 | Solvent assisted flavor evaporation

In the solvent assisted flavor evaporation (SAFE) procedure, odor-active components are distilled under high vacuum. SAFE extraction allows for effective volatile isolation at low temperatures and pressures, thus avoiding potential loss of labile aroma compounds or artifact formation [48]. However, SAFE is both time consuming and labor intensive. SAFE has been previously considered to be the "gold standard" for flavor extraction, so it has been employed for the extraction of VOC in a wide range of foods [48–50]. It is widely used on GC-O analysis because it can be undertaken at low temperature to avoid the creation of thermal volatile aroma-active artifacts [51].

As far as the authors can determine little or no published work exists in relation to DE, LLE or SAFE on beef. Studies using LLE in pork [52, 53] and SAFE in stewed pork [54], heat treated sausages [55] and dry fermented sausages [56] exist. Only SDE has been used in heated beef [57], as well as in chicken [58] and chicken breast meat [59].

4.2 | Sorptive extraction techniques

4.2.1 | Solid-phase microextraction

SPME can be employed as a headspace (HS) extraction technique (HS-SPME) or by direct immersion (DI-SPME). In DI-SPME the SPME fiber is inserted into a liquid sample matrix [60] in a HS vial under controlled conditions. This approach has limited appeal for VOC analysis in foods due to significant reductions in fiber longevity and the expense of the fibers. However, arguably HS-SPME is the most globally used VOC extraction technique for food research today. The underlying principle of HS-SPME is based on the partitioning of analytes between a coated fiber with appropriate stationary phase(s) and the sample [1]. A sample is placed in a sealed HS vial, heated and agitated under controlled conditions until the VOC reach an equilibrium (sometimes referred to HS saturation). At this point a HS-SPME needle is pierced through the vial septum and the fiber is exposed to the gaseous phase in the vial. The next step involves partitioning of the VOC between the sample matrix and the extraction phase(s). The parameters (sample to HS ratio, temperature, time, and degree of agitation) of fiber exposure to the HS has a huge impact on the efficiency of the extraction, mainly as the sorptive process is exothermic [61]. A main advantage of SPME is the wide range of commercial fiber phases available; apolar polydimethylsiloxane (PDMS) for the extraction of non-polar analytes; more polar carbowax-polyethylene glycol (PEG), polyacrylate (PA) and dual phases such as PDMS–divinylbenzene (PDMS-DVB) for the extraction of polar analytes (especially phenols and amines, respectively); Carboxen–PDMS (CAR-PDMS) for the extraction of volatile/low molar mass analytes; Carbowax–DVB (CW DVB) and CW–template resin (CW-TPR) for the extraction of polar analytes (especially alcohols in the first case); and the more generic triple phase fiber DVB-CAR-PDMS for the extraction of a broad range of analytes [60]. In addition, some phases are available at different thicknesses which can benefit the extraction of some VOC. Once the VOC are extracted, the next step involves desorption of VOC from the fiber in the heated inlet of the GC [1]. HS-SPME only requires a small sample size/volume and is suitable for the extraction and concentration of a high number of VOC and semi-VOC (SVOC) [62]. A major benefit of HS-SPME is that it can be fully automated and is relatively easy to use and reproduce [63]. Volatile and non-polar compounds are extracted faster than SVOC and polar volatiles. Some of the drawbacks of HS-SPME are that stationary phases only cover the scale of polarity of target analytes, or certain molecular weight ranges depending upon the phases used. The fibers can be easily damaged (stripping of coatings, bending of the needle) and are relatively expensive [64]. Furthermore, high molecular weight compounds cannot be analyzed in combination with GC, and in certain cases, very volatile, polar, or thermally unstable analytes are reported to have low extraction efficiencies [65]. Another major issue relates to potential analyte displacement. Very volatile compounds are absorbed first and can saturate the fiber due to the relative low capacity of the fibers; also, volatiles with a lower affinity can be displaced over incubation by less volatile compounds with a high affinity for the fiber [65]. In an attempt to circumvent this issue, SPME arrow was developed. SPME arrow uses a larger amount of sorbent material to improve detection limits and is less fragile than the traditional SPME fibers [66]. Another development thin-film SPME aims to increase the volume of the extraction phase and therefore the sensitivity of the method by using a thin extraction phase with a large surface area [67]. The VOC responsible for the aroma of pork products, such as fermented sausages [51, 63–75], frankfurters [68], chorizo [69], salami [70], ham [79–85], pig muscle [39], and porcine liver pates [78] have been extensively studied by HS-SPME. HS-SPME has also been successfully applied in the extraction of VOC in raw beef [79–82], roasted and boiled beef [83], cooked beef [57, 84, 85], minced beef [86]. The different HS-SPME parameters used to determine the aroma-relevant VOC in pork and beef products are described in Tables 3 and 4.

TABLE 3 Static headspace conditions used to determine the profile of aroma-relevant volatile compounds in meat products

	Dried sausages (Salame Milano) [127]	Frankfurters [68]	Raw ham [71]
Instrument parameters			
Sample quantity (g)	20	50	1
Carrier gas	Nitrogen	Nitrogen	Helium
Valve/vial oven temp (°C)	—	—	40
Sample temp (°C)	42	70	—
Sample equil time (min)	30	30	80
Purge temp (°C)	42	—	—
Purge time (min)	30	—	8
Purge flow (ml/min)	50	50	—
Needle temp (°C)	—	—	75
Trap temp (°C)	—	—	40 low; 280 high
Trap hold time (min)	—	—	5
Trap material	Tenax	—	AirToxic™
Desorb temp (°C)	200	250	—
Desorb time (min)	—	5	0.4
Cold trap (°C)	250	—	—
Transfer line to GC temp (°C)	200	—	120
Pressurize (kPa)	—	—	220
Injector temp (°C)	—	—	150
GC-MS parameters			
Column			
Type	DB-1701 column	CPWax 52CB capillary column CPSil 8CB capillary column	Rtx®-200 fused silica capillary column coated with trifluoropropyl methyl polysiloxane stationary phase
Length (m)	30	50	30
Internal diameter (mm)	0.25	0.32	0.32
Film thickness (μm)	1	—	1
Flow rate	—	—	—
Pressure (kPa)	—	–	70
Oven program	35°C for 1 min, raised to 175°C at 4°C/min, to 250°C at 10°C/min held for 5 min	60°C for 5 min, raised to 220°C at 4°C/min	40°C for 5 min, raised to 80°C at 10°C/min held for 3 min; to 180°C at 10°C/min held for 3 min; to 240°C at 15°C/min held for 5 min
Inlet	—	—	Split flow 15 ml/min
MS			
Transfer line to MS temp (°C)	280	—	150
Source temp (°C)	—	—	240
Ionization potential (eV)	70	—	70
Scan range (m/z; amu)	33–300	—	35–300
Scan time (s)	—	—	0.3
Interscan delay (s)	—	—	0.05

Amu, atomic mass units; m/z, mass-to-charge ratio.

TABLE 4 Solid-phase microextraction used to determine the profile of aroma-relevant volatile compounds in meat products

	Fermented sausages [156]	Fermented sausages [157, 158]	Fermented sausages [159]	Fermented sausages [160, 161]	Fermented sausages [162]	Fermented sausages [163]	(Dry-) Fermented sausages [164, 165]	Dry-fermented sausage Sucuk [166]	Dry-fermented sausages [167]
Instrument parameters									
Sample amount (g)	3	3	7 ml	5	3	5	3	5	5
Septum type	PTFE	PTFE	–	PTFE	PTFE	PTFE	—	PTFE	PTFE
Fiber type	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	CAR/PDMS	PDMS; CAR/PDMS; DVB/CAR/PDMS
Fiber film thickness (μm)	85	85	85	85	—	—	85	75	100/85/50-30
Fiber precondition temp (°C)	—	—	—	—	—	—	—	—	—
Fiber precondition time (min)	—	—	—	—	—	—	—	—	—
Sample equil temp (°C)	30	30	37	37	35	37	37	30	37
Sample equil. Time (min)	60	60	15	30	30	30	60	60	30
Fiber exposure time (min)	180	180	120	180	30	120	180	120	180
GC-MS parameters									
Purge valve mode	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless
Desorb temp (°C)	220	240	220	240/250	220	240	220	250	250
Desorb time (min)	15	6	15	5/15	1	5	15	6	15
Carrier gas	Helium	Helium	Helium	–	Helium	—	—	Helium	Helium
Column									
Type	DB-624 capillary column	DB-624 capillary column	DB-624 capillary column	DB-624 capillary column	Cp-wax 52CB capillary column	DB-624 capillary column	DB-624 capillary column	DB-624 capillary column	DB-624/HP-INNOWAX column
Length (m)	30	30	3	30	50	—	30	30	30/30
Internal diameter (mm)	0.25	0.25	0.25	0.25	0.32	—	0.25	0.25	0.25/0.25
Film thickness (μm)	1.4	1.4	1.4	1.	1.2	—	1.4	1.4	1.4/0.25
Flow rate (cm/s)	27.3	27.3	34.3	—	—	—	—	—	—
Pressure (psi)	—	—	—	—	—	—	—	—	—

(Continues)

TABLE 4 (Continued)

	Fermented sausages [156]	Fermented sausages [157, 158]	Fermented sausages [159]	Fermented sausages [160, 161]	Fermented sausages [162]	Fermented sausages [163]	(Dry-) Fermented sausages [164, 165]	Dry-fermented sausage Sucuk [166]	Dry-fermented sausages [167]
Oven program	38°C for 13 min, raised to 110°C at 3°C/min, to 150°C at 4°C/min, to 210°C at 10°C/min held for 5 min	38°C for 13 min, raised to 110°C at 3°C/min, to 150°C at 4°C/min, to 210°C at 10°C/min held for 5 min	38°C for 13 min, raised to 100°C at 3°C/min, to 150°C at 4°C/min, to 210°C at 10°C/min held for 5 min	38°C for 13 min, raised to 110°C at 3°C/min, to 150°C at 4°C/min, to 210°C at 10°C/min held for 5 min	40°C for 2 min, raised to 200°C at 10°C/min, to 250°C at 15°C/min held for 5 min	—	38°C for 13 min, raised to 110°C at 3°C/min, to 150°C at 4°C/min, to 210°C at 10°C/min held for 5 min	40°C for 5 min, raised to 110°C at 3°C/min, to 150°C at 4°C/min, to 210°C at 10°C/min, held for 15 min	38°C for 13 min, raised to 110°C at 3°C/min, to 150°C at 4°C/min, to 210°C at 10°C/min held for 5 min
Detector type	MSD	MSD	—	FID	FID	—	FID	MSD	FID
MS						—			
Transfer line temp (°C)	240	240	240	—	—	—	—	24	240
Ion source temp (°C)	—	—	230	—	—	—	—	—	—
Ionization potential (eV)	70	70	70	70	—	—	70	7	70
Scan range (amu)	29–40	29–400	29–400	29–400	—	—	29–400	50–500	29–400
Scan speed	—	—	—	—	—	—	—	—	—

	Pork fermented sausages [168]	Dry-ripened chorizo [69]	Salami [70]	White dry-cured ham [72]	Dry-cured ham [73]	(Serrano) Dry-cured ham [74, 75]	Iberian ham [76]	Istrian dry-cured ham [77]	Porcine liver pate [78]
Instrument parameters									
Sample amount (g)	50 µl	2	1	3	3	10	3	10 ml	1
Septum type	–	Teflon-rubber	Teflon-rubber	PTFE	PTFE	PTFE	PTFE	PTFE	–
Fiber type	DVB/CAR/P	DVB/CAR/P	CAR/PDMS	DVB/CAR/P	DVB/CAR/P	DVB/CAR/P	DVB/CAR/P	DVB/CAR/PDMS	DVB/CAR/PDMS
Fiber film thickness (µm)	50/30	50/3	75	50/30	50/30	50/30	—	50/30	50/30
Fiber precondition temp (°C)	270	270	300	270	270	—	—	240	220
Fiber precondition time (min)	30	60	60	60	60	—	—	2	45
Sample equil temp (°C)	40	35	35	40	40	35	40	40	60

(Continues)

TABLE 4 (Continued)

	Pork fermented sausages [168]	Dry-ripened chorizo [69]	Salami [70]	White dry-cured ham [72]	Dry-cured ham [73]	(Serrano) Dry-cured ham [74, 75]	Iberian ham [76]	Istrian dry-cured ham [77]	Porcine liver pate [78]
Sample equil. Time (min)	—	15	15	10	10	60	10	—	30
Fiber exposure time (min)	90	30	30	180	180	60	180	180	—
GC-MS parameters									
Purge valve mode	Splitless; 50 ml/min for 2 min	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless	Splitless
Desorb temp (°C)	260	260	250	260	260	260	260	230	220
Desorb time (min)	5	5	5	5	5	10	5	2	–
Injection port temp (°C)	—	260	250						220
Carrier gas	Helium	Helium	—	Hydrogen	Hydrogen/H	Helium	Hydrogen/H	Helium	Helium
Column									
Type	DB-WAX polar column	DB-624 capillary column	CP-Sil 8 CB low bleed/MS fused silica capillary column	DB-WAX column	DB-WAX column	Zebron 100% polyethylene glycol capillary column	DB-WAX column	DB-5 ms capillary column	5% phenyl-95% dimethyl polysiloxane column
Length (m)	60	30	60	60	60	60	60	30	30
Internal diameter (mm)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Film thickness (μm)	0.25	1.	0.25	0.25	0.25	0.50	0.25	0.25	1
Flow rate (ml/min)	1	40 cm/s	—	—	—	—	—	1	1.6
Pressure (psi)	—	—	—	—	15	15	15	—	18.5
Oven program	40°C for 3 min, raised to 150°C at 3°C/min, to 220°C at 7°C/min held for 5 min	40°C for 10 min, raised to 200°C at 5°C/min, to 250°C at 20°C/min held for 5 min	40°C for 2 min, raised to 280°C at 4°C/min held for 5 min	40°C for 4 min, raised to 91°C at 1°C/min, to 201°C at 10°C/min held for 10 min	40°C for 4 min, raised to 91°C at 1°C/min, to 201°C at 10°C/min held for 10 min	45°C for 16 min, raised to 110°C at 4°C/min held for 9 min, to 230°C at 15°C/min held for 3 min, to 250°C at 10°C/min held for 2 min	40°C for 4 min, raised to 91°C at 1°C/min, to 201°C at 10°C/min held for 10 min	40°C for 10 min, raised to 200°C at 5°C/min, to 250°C at 20°C/min held for 5 min	40°C for 10 min, raised to 250°C at 7°C/min, held for 5 min

(Continues)

TABLE 4 (Continued)

	Pork fermented sausages [168]	Dry-ripened chorizo [69]	Salami [70]	White dry-cured ham [72]	Dry-cured ham [73]	(Serrano) Dry-cured ham [74, 75]	Iberian ham [76]	Istrian dry-cured ham [77]	Porcine liver pate [78]
Detector type	—	MSD	—	FID	FID	—	FID	—	FID
MS			—						
Transfer line temp (°C)	—	—	—	—	—	280	—	280	270
Ion source temp (°C)	230	—	—	—	—	230	—	—	—
Ionization potential (eV)	70	70	70	—	—	70	—	70	70
Multiplier voltage (V)	—	1953	—	—	—	—	—	—	1650
Scan range (*m/z*; amu)	45–550	40–300	—	—	—	33–280	—	50–450	40–300
Scan speed (scan/s)	—	6.34	—	—	—	1.74	—	1	1

	Pig muscle [169]	Duroc-Bamei pig [170]	Cattle beef [85]	Hanwoo beef [84]	Raw red meat [82]
Instrument parameters					
Sample amount (g)	6	5 ml	1	1	2
Septum type	PTFE	—	PTFE	PTFE	Teflon-silicone
Fiber type	PDMS/Polyacrylate	—	CAR/PDMS	CAR/PDMS	DVB/CAR/PDMS
Fiber film thickness (μm)	100/85		—	75	50/30
Fiber precondition temp (°C)	—	250	250	250	—
Fiber precondition time (min)	—	10	10	10	—
Sample equil temp (°C)	80	55	60	60	50
Sample equil. time (min)	40	15	10	10	—
Fiber exposure time (min)	40	20	30/45/60	60	30
GC-MS parameters					
Purge valve mode	—	—	Split ratio 10:1; split flow 10 ml/min	Split ratio 10:1; split flow 10 ml/min	—
Desorb temp (°C)	250	—	250	250	250
Desorb time (min)	—	—	5	5	5
Injection port temp (°C)	—	240	25	250	—
Carrier gas	Helium	Helium	Helium	Helium	Helium
Column					
Type	PEG capillary column	TG-5MS capillary column	DB-5 ms capillary column	DB-5 ms capillary column	DB-WAX capillary column
Length (m)	30	30	30	30	30

(Continues)

TABLE 4 (Continued)

	Pig muscle [169]	Duroc-Bamei pig [170]	Cattle beef [85]	Hanwoo beef [84]	Raw red meat [82]
Internal diameter (mm)	0.25	0.25	0.25	0.25	0.25
Film thickness (μm)	0.25	0.25	0.25	0.25	0.25
Flow rate (ml/min)	0.8	1	1	1	1.2
Pressure (psi)	—	—	7.03	7.03	—
Oven program	35°C for 5 min, raised to 230°C at 5°C/min	50°C for 2 min, raised to 240°C at 4°C/min held for 5 min	40°C for 5 min, raised to 250°C at 8°C/min held for 5 min	40°C for 5 min, raised to 250°C at 8°C/min held for 5 min	50°C for 4 min, raised to 145°C at 10°C/min held for 6 min, raised to 160°C at 10°C/min held for 3 min, then 200°C at 10°C/min held for 3 min
Detector type	—	—	—	—	MSD
MS					
Transfer line temp (°C)	–	250	250	250	150
Ion source temp (°C)	200	250	—	—	230
Ionization potential (eV)	70	70	70	70	70
Multiplier voltage (V)	—	—	—	—	—
Scan range (*m*/*z*; amu)	30-200	40-450	35-300	35-300	30-400
Scan speed (scan/s)	—	0.3	5.27	5.27	—

Amu, atomic mass units; DVB/CAR/PDMS, divinylbenzene/carboxen/polydimethylsiloxane; FID, flame ionization detector; MSD, mass selective detector; *m*/*z*, mass-to-charge ratio; PTFE, polytetrafluorethylene.

4.2.2 | Stir bar sorptive extraction

Stir bar sorptive extraction (SBSE) is a solventless sample preparation method for the extraction and enrichment of VOC from aqueous matrices. The method is based on sorptive extraction, whereby the solutes are extracted into a polymer coating on a magnetic stirring rod. The extraction is controlled by the partitioning coefficient of the solutes between the polymer coating and the sample matrix and by the phase ratio between the polymer coating and the sample volume [87]. For SBSE, it is known that the analyte recovery in the extraction phase is high. Moreover, this method provides enhanced sensitivity because the extracted fraction can be introduced quantitatively into a GC system by TD. However, extraction of polar/hydrophilic solutes at the trace level is still a serious limitation because this method is generally more selective toward hydrophobic/apolar solutes [88]. Applications of SBSE in food analysis are limited; analysis of VOC in strawberries [89], grapes [90], coffee [91], beer [92], wine [93], whiskey [94], sake [95], and vinegar [96]. To date only two studies have been undertaken on beef or pork; grilled beef [97, 98] and one on cooked ham [99].

4.2.3 | High capacity sorptive extraction

High capacity sorptive extraction (Hi-Sorb) is relatively new and innovative, semi-automative approach for the analysis of VOC by TD GC-MS. Hi-Sorb probes are able to sorptively extract VOC and SVOC from a range of sample types, both from the HS or by DI of liquids, similar to SPME or SBSE. This is an easy-to-use, high capacity highly sensitive approach without the expense of time associated with solvents extraction methods. Unattended sample preparation using the Hi-Sorb Agitator, or with a semi-automated or fully automated system such as Centri (Markes International, Bridgend, UK) in tandem with a TD, ensures maximum productivity, while reusable probes and tubes minimize the cost per sample. It is highly reproducible and takes high sample capacity. Hi-Sorb in combination with TD enables venting of unwanted analytes, VOC enrichment, and recollection. Depending upon the degree of automation, it can be relatively expensive but the probes are reusable. Initially it was only available with PDMS but is now also available with PDMS/CWR, PDMS/DVB, and DVB/CWR/PDMS. Typical Hi-Sorb applications include aromatics in coffee, lactones in milk, bacterial VOC in bodily fluids and esters in whisky [100], and whole milk powder [101], but no research has been carried out on meat to date.

4.3 | Dynamic headspace extraction techniques

DHS is one of the most widely used methods for volatile fraction analysis [42]. DHS transfers VOC from a solid or liquid matrix to the vapor phase by heating in a controlled inert gas flow. The carrier gas is either swept over the sample surface or bubbled through the sample [102]. The concentration and volatility of the analytes, sample size, sorbent type, extraction time, and carrier gas flow determine the effectives of the extraction [42]. When a sample presents analytes at ultra-trace levels in the parts-per-billion (ppb) to parts-per-trillion (ppt) range, or where an exhaustive extraction of the analyte is required [103], DHS is particularly useful [42] as the capacity of the sorbent phase in relation to the sample mass/volume is high. DHS offers many advantages: easy sample preparation, complete automation, solvent-less, eliminates contamination, and a wide range of sorbent phases are available [104]. It is also possible to vent unwanted analytes, enrich extracts and recollect extracts. Overall, the potential to optimize this system to target specific analytes is large [5]. However, the main issue with DHS is moisture control. DHS systems can manage moisture control via dry purging but in samples with significant levels of moisture it is more problematic, as moisture if introduced to the GC column can adversely impact its lifespan, and increase background noise through phase deterioration and damage the MS-detector [5]. DHS has been used to determine distinct aroma flavors of pork sausages [105–107], frankfurters [68], ham [108, 109] and *lacon* [110], as well as cooked beef [111, 112]. The conditions under which these foods were analyzed are listed in Table 5.

4.3.1 | Purge and trap

P&T was the method of choice for extracting and concentrating VOC from almost any matrix [113] prior to the advent of easier to use automatable techniques, especially sorptive extraction techniques. P&T is a DHS sampling technique where the inert gas is passed through the sample onto a trap containing adsorbent or absorbent materials which retain the VOC [5]. The trap is rapidly heated to desorb the VOC which are then transferred to the GC column [113]. The main advantages of P&T are enhanced sensitivity for highly volatile compounds, the absence of extended heating times, and reproducibility [114]. The disadvantages are mainly related to moisture management, control of foaming, the semi-automotive approach, and VOC carry over. P&T has been used for the analysis of raw beef [115], cooked beef [34, 116, 117], and grilled beef [98], but also

TABLE 5 Dynamic headspace conditions used to determine the profile of aroma-relevant volatile compounds in meat products

	Dry fermented sausages [105]	Irradiated sausages [106]	Frankfurters [68]	Seasoned sausages (salami) [107]	Dry-cured Iberian ham [108]	Jinhua ham [109]	Dry-cured lacon [110]	Hamburger patty [111]	Korean cattle, Holsteins, Angus steers [112]
Instrument parameters									
Sample amount (g)	10	1	50	—	8	—	10	1	10
Carrier gas	Helium	Helium	Nitrogen	—	Helium	Nitrogen	Helium	—	—
Sample temp (°C)	—	—	70	—	40	50	60	—	85
Preheat time (min)	—	—	30	—	10	30	5	5	30
Purge temp (°C)	—	40	—	40	35	—	—	52-53	50°C for 3 min, raised to 240°C at 5°C/min held for 9 min
Purge time (min)	45	15	—	206	30	20	20	15	—
Purge flow (ml/min)	40	40	50	—	—	100	60	—	—
Trap temp (°C)	—	—	—	—	—	—	—	20	—
Trap time (min)	—	—	—	—	—	—	—	—	—
Trap material	Tenax	Tenax/silica/charcoal	Tenax	—	Tenax	Tenax	Tenax	—	—
Desorb temp (°C)	220	220	—	—	225	280	225	185	—
Desorb time (min)	—	1	—	—	2	—	—	—	—
Desorb flow rate (ml/min)	—	—	—	—	—	—	300	—	—
Transfer line to GC temp (°C)	—	—	—	—	200	—	—	—	—
Injector temp (°C)	—	—	—	250	—	250	220	—	—
Detector temp (°C)	—	—	—	250	280	—	260	250	—
GC-MS parameters									
Carrier gas	Helium	Helium	—	Helium	—	Helium	Helium	—	Helium
Column									
Type	DB-5 apolar capillary column	HP-Wax; HP-5	CPWax 52CB; CPSil 8CB capillary column	DB-Wax fused capillary column	DB-5 fused Silica capillary column	DB-5 ms UI DB-WAX	DB-624 capillary column	DB-624 megabore capillary column	HP-PLOT Q

(Continues)

TABLE 5 (Continued)

	Dry fermented sausages [105]	Irradiated sausages [106]	Frankfurters [68]	Seasoned sausages (salami) [107]	Dry-cured Iberian ham [108]	Jinhua ham [109]	Dry-cured lacon [110]	Hamburger patty [111]	Korean cattle, Holsteins, Angus steers [112]
Length (m)	60	7.5; 30	50	30	50	30	30	75	30
Internal diameter (mm)	0.33	0.25; 0.25	0.32	0.25	0.32	0.25	0.25	0.53	0.53
Film thickness (μm)	—	0.25; 0.25	—	0.5	1.05	0.25	1.4	3	0.25
Flow rate (ml/min)	1.2	—	—	1	—	1.2	36 cm/s	—	20
Oven program	40°C, raised to155°C at 3°C/min	7°C for 2.5 min, raised to 25°C at 3°C/min, to 120°C at 10°C/min, and to 200°C at 20°C/min	60°C for 5 min, raised to 220°C at 4°C/min	35°C for 5 min; raised to 80°C at 2°C/min; to 200°C at 4°C/min	35°C for 10 min; raised to 150°C at 7°C/min; to 250°C at 20°C/min held for 5 min	40°C for 3 min, raised to 200°C at 5°C/min, to 230°C at 5°C/min held for 3 min	40°C for 2 min, raised to 100°C at 3°C/min, to 180°C at 5°C/min, to 250°C at 9°C/min held for 5 min	40°C for 30 min, raised to 80°C at 10°C/min, to 220°C at 4°C/min held for 20 min	35°C for 5 min, raised to 180°C at 7°C/min, raised to 250°C at 5°C/min held for 21 min
Inlet	Injection split 1 ml/min	—	—	Splitless	—	Splitless; Inlet heating rate 12°C/min to 260°C	Split ratio 1:20	—	210°C
MS									
Transfer line to MS temp (°C)	—	155	—	—	280	280	—	—	250
Ionization potential (eV)	70	70	70	—	—	70	70	—	—
Scan range (*m*/*z*; amu)	—	46.1–550	35–250	—	—	45–650	40–300	—	30–250
Scan speed (scan/s)	—	2.94	—	—	—	—	6.34	—	0.2
Quadrupole temp (°C)	—	—	—	—	—	—	—	—	—
Ion source temp (°C)	—	—	—	—	—	230	—	—	250

mu, atomic mass units; *m*/*z*, mass-to-charge ratio.

for pork sausages [118–120] and ham [121, 122]. The extraction/concentration conditions of the P&T technique used to determine the volatile profile of the meat products are shown in Table 6.

4.3.2 | Thermal desorption

Thermal desorption (TD) is a common DHS technique, frequently used for VOC and SVOC analysis. In its simplest form, TD involves heating a sample and collecting VOC in a tube packed with absorbent and adsorbent phases in a gas stream. Like P&T the tube is desorbed directly to the GC. In summary volatiles are desorbed from the sample and introduced in the GC by an online process, thus saving time, sample handling, and reagents. In many TD applications a focusing trap is present inline prior to transfer to the GC, to reduce peak broadening and further enrich the volatiles as multiple tubes can be collected into the same focusing trap. Focusing traps are often made of the same phases as TD tubes and therefore different options are also available to discriminate or concentrate as desired. TD also has a very low risk of sample contamination because a minimal manipulation of the sample is required [123]. Many TD systems enable re-collection, running blanks, automated leak detection with a very high degree of control over gas flow, backflushing, pressure, and temperature control. This technique has been used for qualitative analysis of VOC and SVOC, as a wide range of phases are available. Recoveries of both thermally labile and low volatility compounds have been found to be effective using TD [124]. TD has not been used extensively in meat products [125], possibly due to moisture control issues, however some studies exist on beef [126], grilled beef [98], and dried sausages [127]. The extraction/concentration conditions of the TD technique used to determine the volatile profile meat products are shown in Table 7.

4.3.3 | Solid-phase dynamic extraction

Solid-phase dynamic extraction (SPDE) is based on the same principles as SPME, but uses an internally coated steel needle instead of a fiber for the extraction and preconcentration of VOC from the sample [128]. SPDE can be used in HS or DI modes. The syringe plunger is moved up and down several times for a dynamic extraction of the sample, and the analytes are sorbed in the internal coating. After a predetermined number of extraction cycles (aspirating and dispensing), the analytes are thermally desorbed from the coating in the GC injector [129]. SPDE offers several inherent advantages over SPME and SBSE, mainly that SPDE needle coatings possess around four to six times larger extraction phase volumes (4.5 µl of the polymer) compared with a 100-µm SPME fiber (0.6 µl of PDMS) [130]. SPDE has been applied to a wide range of target compounds and different food matrices [131]. One study [132] utilized SPDE to quantify pyrazines in barbecued beef patties using PDMS and activated carbon, but otherwise very little studies appear to have used this extraction technique for the analysis of volatile compounds in beef or pork. This may be due to difficulties in preventing moisture ingress into the needle that may adversely impact VOC interaction with the phase, and/or optimizing the extraction methods in relation to number of pump strokes and volume, extraction temperatures, times, and desorption.

5 | GAS CHROMATOGRAPHY

GC is a common type of chromatography used to separate and analyze VOC, SVOC, or even some non-volatile compounds by derivatization [133]. Nowadays, GC continues to play an important role in the analysis of numerous food components [134]. It is always necessary to include a prior step involving the extraction and pre-concentration of the volatile fraction [123]. Once the VOC have been isolated from their matrix, their analysis is carried out by GC with an appropriate detector. A variety of columns with different properties are available. The use of fused-silica capillary columns with improved surface inertness, thermal stability and resolution best fulfils most of the analysis requirements (good resolution, high repeatability and reproducibility, high precision, minimum decomposition) [135]. The selection of the proper capillary column for any application should be based on four significant factors which are stationary phase (ranging from nonpolar to polar or highly polar), column internal diameter, film thickness, and column length. Each stationary phase provides a specific combination of interactions for each chemical class of analytes. A non-polar column is best for the analyses of non-polar compounds, generally composed only of carbon and hydrogen atoms and contain carbon-carbon single bonds. Polar columns most effectively separate polar compounds, composed primarily of carbon and hydrogen atoms, but also contain one or more atoms of bromine, chlorine, fluorine, nitrogen, oxygen, phosphorus, or sulfur [136]. Hydrogen, helium, or nitrogen can be used as carrier gas, however traditionally helium is favored as it provides a faster flow than nitrogen and the flammability of hydrogen restricted its use [5]. However, as helium gets more expensive as supplies dwindle, hydrogen usage continues to rise as it is relatively inexpensive when generated in-house and the built-in safety devices in hydrogen generators and in GCs significantly reduce the hazard.

TABLE 6 Purge and trap conditions used to determine the profile of aroma-relevant volatile compounds in meat products

	Dry-fermented sausages [118]	Dry-fermented sausages [119]	Seasoned sausages (salami) [120]	Dry-cured ham [121]	Iberian ham [122]	Raw beef [115]	Cooked beef (bull and heifer) [116]	Cooked beef (cattle) [117]
Instrument parameters								
Sample amount (g)	25	1	10	1	6	15	10	10
Carrier gas	Nitrogen	Nitrogen	Helium	Nitrogen	Helium	Helium	Helium	Helium
Sample temp (°C)	30	45	70	35	50	30	70	70
Preheat time (min)	30	5	15	30	5	—	—	—
Purge temp (°C)	30	—	—	—	-20	30	—	—
Purge time (min)	60	10	—	—	30	16	10	10
Purge flow (ml/min)	40	43	—	—	40	40	40	40
Trap temp (°C)	—	—	240	—	220	—	30	30
Trap time (min)	—	—	—	—	2	—	—	—
Trap material	Tenax	Tenax/silica gel/charcoal	—	—	Tenax/silica gel/charcoal	Tenax	Tenax	Tenax
Desorb temp (°C)	—	225	—	150	220	180	180	180
Desorb time (min)	5	2	—	20 s	—	4	4	4
Desorb flow rate (ml/min)	—	—	—	—	—	40	40	40
Transfer line to GC temp (°C)	—	—	—	—	210	—	—	—
Injector temp (°C)	—	210	—	—	230	250	150	150
Detector temp (°C)	—	220	—	—	250	—	250	—
GC-MS parameters								
Carrier gas	—	Helium	—	Helium	Helium	Helium	Helium	Helium
Column								
Type	CP-Sil 8 CB low bleed/MS fused silica capillary column	DB 23 column	PS 264 capillary fused-silica column	DB-624 column	HP-5 fused capillary column; HP-FFAP fused capillary column	HP-5 capillary column	HP-5 capillary column	HP-5 capillary column
Length (m)	60	30	50	30	50/30	50	50	50

(Continues)

TABLE 6 (Continued)

	Dry-fermented sausages [118]	Dry-fermented sausages [119]	Seasoned sausages (salami) [120]	Dry-cured ham [121]	Iberian ham [122]	Raw beef [115]	Cooked beef (bull and heifer) [116]	Cooked beef (cattle) [117]
Internal diameter (mm)	0.25	0.32	0.32	0.25	0.32	0.32	0.32	0.32
Film thickness (μm)	0.25	0.25	3	1.4	1.05/0.25	1.05	1.05	1.05
Flow rate (ml/min)	—	—	—	1	—	1	1	1
Pressure (psi)	—	70 kPa	—	—	—	6	10	10
Oven program	40°C for 2 min, raised to 280°C at 4°C/min held for 5 min	35°C for 8 min, raised to 220°C at 10°C/min	40°C for 6 min, raised to 180°C at 5°C/min held for 5 min, to 200°C at 7°C/min held 2 min, to 240°C at 7°C/min held 5 min	50°C for 5 min, raised to 225°C at 5°C/min held for 10 min	35°C for 5 min, raised to 150°C at 10°C/min, to 250°C at 20°C/min held for 10 min	35°C for 15 min, raised to 220°C at 8°C/min held for 15 min	35°C for 15 min, raised to 220°C at 8°C/min held for 5 min	35°C for 15 min, raised to 220°C at 8°C/min held for 5 min
Inlet	—	—	—	Split ratio 1:25 at 150°C	—	Split ratio 5:1	Split ratio 1:5	Split ratio 1:5
MS								
Transfer line to MS temp (°C)	—	—	250	225	280	—	—	—
Ionization potential (eV)	70	—	70	70	70	70	70	70
Multiplier voltage (V)	—	—	—	—	1675	2000	3000	2000
Scan range (m/z; amu)	—	—	29–300	35–350	30–300	30–250	30–250	30–250
Scan speed (scan/s)	—	—	0.5	1	1	3.32	3.32	3.32
Quadrupole temp (°C)	—	—	—	—	—	108	108	108
Ion source temp (°C)	—	—	—	—	—	230	230	230

Amu, atomic mass units; m/z, mass-to-charge ratio.

TABLE 7 Direct thermal desorption conditions used to determine the profile of aroma-relevant volatile compounds in meat products

	Dried sausages (Salame Milano) [127]	Grilled beef [98]
Instrument parameters		
Sample quantity (g)	20	Stir bars
Carrier gas	Nitrogen	Helium (1.2 ml/min)
Sample temp (°C)	42	
Sample equil time (min)	30	
Purge temp (°C)	42	
Purge time (min)	30	
Purge flow (ml/min)	50	
Trap material	Tenax	
Desorb temp (°C)	200	40°C for 1 min, 200°C for 5 min
Cold trap (°C)	250	
Transfer line to GC temp (°C)	200	
GC-MS parameters		
Column		
Type	DB-1701 column	HP-5 MS fused-silica capillary column
Length (m)	30	30
Internal diameter (mm)	0.25	0.25
Film thickness (μm)	1	0.25
Oven program	35°C for 1 min, raised to 175°C at 4°C/min, to 250°C at 10°C/min held for 5 min	50°C for 1 min, raised to 100°C at 10°C/min held for 2 min, to 280°C at 30°C/min held for 1 min
MS		
Transfer line to MS temp (°C)	280	
Ionization potential (eV)	70	70

6 | IDENTIFICATION OF VOLATILES—DETECTION SYSTEMS

GC can be used with a wide range of ionization detectors, the most common are flame ionization detectors (FID), electron capture detectors (ECD), or thermal ionization detectors (TID). However, for VOC analysis the preferred choice, by far, is coupling GC with MS detectors. Although relatively expensive compared to ionization detectors, it is imminently suited as gas flows are complementary, the systems are easily controlled/managed, sample extracts for volatile analysis are relatively clean, and information on compound identity using mass to charge ratio and retention times are easily achieved [133]. Different mass ionization processes exist, but electron ionization at 70 V is the most common. The main advantages of 70 V mass ionization energy are the wide range of commercial mass libraries of volatile compounds that are available, which aids identification of VOC. The types of mass analyzers widely available for GC-MS today, include single-quadrupole (Q) MS, time-of-flight (TOF) MS, triple-quadrupole (TQ) MS, and high-resolution systems such as, QTOF MS and Orbitrap MS. High-resolution MS systems (QTOF and Orbitrap) are becoming more popular due to their higher scanning speeds and increased sensitivity and dynamic range. However, due to costs, most volatile analysis of foods is still undertaken using Q MS. The main disadvantages of GC-MS in general remain the costs, but also the time required and the need for experienced highly trained operatives to maximize the potential of this hyphenated technique [103, 133].

The compounds can be identified (a) by comparison with commercial reference standards [137], (b) by comparison of Kovats indices with those described by other authors [138], and (c) by comparison of their mass spectra with those contained in libraries, such as NBS (National

Bureau of Standards) [139], Wiley [137], NIST/EPA/NIH [140], FFNSC 3 [141], MassLab v.1.3 [73], Aroma Office ^{2}D [142], mzCloud mass spectral library [143] or in-house libraries constructed using target compounds.

7 | GAS CHROMATOGRAPHY OLFACTOMETRY

The extraction techniques as mentioned above in combination with GC-MS can help researchers to identify the VOC in meat samples but they cannot identify the aroma characteristics of the VOC involved, nor identify those impacting sensory perception, or their perceived intensity. This can only be achieved via GC-O. GC-O utilizes the human nose as an additional detector in combination with mass spectrometry or flame ionization detection [144].

Experienced trained assessors describe each perceived odor, and can provide additional information on its relative intensity [145]. There are three main GC-O methods used in the characterization of odor-active compounds, which are based on the principle of detection frequency, time intensity, and dilution to threshold [144]. Detection frequency provides the number or percentage of individuals who sensed the odor for a compound [144]. Time-intensity methods provide data on the description and intensity of the odor evaluated using a quantitative scale [144]. Dilution to threshold consists of preparing a series of sample dilutions and each dilution is analyzed until no odorant could be detected [144]. The main disadvantages are that only aromas from individual or co-eluting VOC can be determined and not the global aroma profile of the sample, the time taken to train panelists, and having to evaluate samples in triplicate with multiple assessors. Co-elutions can hide the true aroma of each single compound, influencing perception. Hence, more novel analytical approaches such as GCxGC may be used [146], but it can be difficult to get sufficient VOC concentration in the second dimension for olfactometry analysis. Synergies with other compounds from the matrix and/or the change of their sensory characteristics by their concentration may also influence the analysis making only a small percentage of volatiles odor-active [5]. Often with GC-O characteristic aromas are detected without any corresponding peak, which highlights the sensitivity of the human nose over the detector. However, combining GC-O with descriptive sensory analysis, in short, applying sensometrics can provide more accurate information in relation to the overall aroma of the food [144]. The volatile profile of cooked meat was assessed by GC-O [147], but it appears that significant scope exists to further utilize this approach to determine factors that influence the sensory characteristic of beef aroma. The choice of VOC extraction method is important in GC-O, as normally you are trying to avoid the creation of odor active thermal artifacts or to lose thermally labile VOC [51]. This is why extraction techniques that use solvents rather than temperature tend to be favored for GC-O. However, in meat applications temperature is needed as you are often trying to elucidate the key flavor compounds in cooked product, hence trying to mimic the cooking parameters used to prepare the product for sensory evaluation.

8 | FUTURE TRENDS

The future trends in GC or GC-MS should continue to focus on flexibility of VOC extraction/concentration, platform miniaturization, increased sensitivity, automation, and better resolution. There is still work to be done around capabilities for increasingly lower detection limits, through more efficient generation and transfer of ions, and in the chromatography process itself. Firstly, high sensitivity is required to ensure that trace compounds are detected because even a trace amount of a compound with a low odor threshold can have a large impact on the overall aroma. Secondly, the diverse range of chemical classes requires advanced separations to resolve co-elutions and provide confident identification of the analytes present. Comprehensive GCxGC with time-of-flight mass spectrometry (TOF MS) or Orbitrap MS can tackle this challenge by coupling two columns of different selectivity to separate the analytes based on two different chemical properties (e.g., volatility and polarity). These detectors feature both high resolution as well as high mass accuracy and can be used to distinguish between VOC with the same nominal mass, determine elemental compositions and identify unknowns. The full automatization of sample preparation, VOC extraction and concentration using multiple phases in tandem with multiple detection channels would help to address the need for more specific and confident results with shorter turnaround times. Finally, more sophisticated data analysis software is required to quickly identify subtle differences between aroma profiles and find associations between aroma compounds and sensory perception.

With respect to beef and pork flavor, consumers are becoming more discerning about quality and production history in addition to price. More research in customizing the beef and pork flavor through production processes and genetics is useful [148–150]. Studies could focus on altering the fatty acid composition of the meat by specialized formula feed with the purpose of manipulating the flavor of the meat. Moreover, the actual trend to demand natural flavor ingredients in foods make flavor scientists to look for

precursors and biotechnology processes for the production of natural meat flavors.

9 | CONCLUDING REMARKS

Beef and pork are reservoirs of VOC that can contribute to the development of complex flavors during cooking. All the volatile components that are responsible for the special flavor of beef and pork appear to fall into the following chemical classes: aldehydes, ketones and alcohols, with some acids, esters, furans and N-/S-containing compounds too [38, 39]. Among them, the impact of sulfur-containing compounds is of main importance in beef and pork aroma, but the contribution of other aroma VOC should not be underestimated due to possible synergies amongst other VOC. The key volatiles of cooked beef include: octanal, nonanal, (E,E)-2,4-decadienal, methanethiol, methional, 2-furfurylthiol, 2-metyl-3-furanthiol, 3-mercapto-2-pentanone, and 4-hydroxy-2,5-dimethyl-3-(2H)-furanone [10]. These compounds occur also in cooked pork differing in concentrations. Other compounds thought to contribute to the flavor of pork are hexanal, 1-nonanal, 3-methyl-1-butanol, 1-penten-3-one, 2-pentyl-furan, N-morpholinomethyl-isopropyl-sulfide and methyl butyrate [28]. Thousands of VOC have been identified in beef and pork, but it is the use of olfactometry methodologies and MS that contribute to increase the knowledge of the importance of these in relation to consumer preference.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

DATA AVAILABILITY STATEMENT

Data sharing not applicable – no new data generated

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Review article

Volatile compounds of six species of edible seaweed: A review

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ABSTRACT

Seaweeds are widely distributed throughout the world. Many seaweeds are of commercial importance with some consumed directly or used as an ingredient as they have functional, nutritional and/or organoleptic properties. Consumer acceptance of food is closely related to its sensory properties, of which flavor is of prime importance. A significant contributor to flavor is the aromatic volatile components present. This review focusses on the volatile components identified in commercially important edible macroalgae species consisting of four brown (*Himanthalia elongata*, *Laminaria* spp. including *L. ochroleuca*, and *Undaria pinnatifida*) and two red species (*Porphyra umbilicalis* and *Palmaria palmata*). In excess of 200 volatile compounds have been identified consisting of hydrocarbons, alcohols, aldehydes, ketones, acids, esters, furans, phenols and sulfur- containing compounds, among others present in minor quantities. The extraction/concentration conditions, chromatography and detection methodologies applied varied and impacted on the volatiles identified due to differences in their hydrophobicity, molecular weight and vapor pressure. This review highlights that considerable more information is required to identify volatile aromatic compounds in edible macroalgae and to identify those most likely to impact sensory perception. Such information could be used to aid new product development or widen applications of these seaweeds in the food or beverage sectors.

1. Introduction

Seaweed or macroalgae are a large diverse group of macroscopic, eukaryotic, photosynthetic, marine organisms. Ten thousand different species have been described to date [1]. According to their pigmentation, seaweeds are classified into three higher taxa, which include brown seaweed (Phaeophyceae), green seaweed (Chlorophyceae), and red seaweed (Rhodophyceae) [2]. Seaweeds compose a credible source of functional ingredients with bioactive and volatile aromatic compounds [3]. There are currently 15–20 species of edible seaweed commercially available for consumption in Europe [4]. Seaweeds are widely consumed, especially in Asian countries, fresh, dried, or as ingredients in prepared foods [5]. They represent a food group that is not normally ingested in unprocessed form in Western societies, where algae remain of minor importance in spite of proven nutritional benefits [6].

Researchers worldwide have placed great importance on the chemical composition and bioactive components in seaweeds [7,8]. Their use as functional ingredients has instigated new developments in food processing [9,10], one of which is in meat product formulation [5]. Seaweeds have significant potential for use in processed foods as they can provide strong odors and characteristic marine flavors [11]. Researchers have also investigated the volatile fraction of some edible seaweed species, generally via gas chromatography mass spectrometry (GC–MS). Volatile compounds from macroalgae can be extracted utilizing several processes, the aim of which is to obtain an aromatic profile of the species in fresh or processed form [12]. The extraction and concentration of volatile compounds from seaweeds has been conducted using headspace solid-phase microextraction (HS-SPME) [13,14], dynamic headspace extraction (DHE) [11] and static headspace extraction (SHE) [15], simultaneous distillation-extraction (SDE) [16], supercritical fluid extraction (SFE) [17], pressurized solvent extraction (PSE) [18], multiple headspace sorptive extraction (MHSSE) [19] and pre-evaporation [20]. It is well established that even though multiple extraction techniques are available for the analysis of volatile organic compounds none as yet can provide a complete volatile profile as each has inherent bias for certain chemical classes based on volatility, polarity, vapor pressure and molecular weight [12].

Consumers' acceptance of food is intimately related to its flavor. Therefore, volatile components are very important parameters in food quality and flavor [11]. Volatile organic compounds are molecules with high vapor pressure, moderate hydrophilicity and low molecular

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weight. Hydrocarbons, ketones, aldehydes, alcohols, carboxylic acids, esters, halogenated compounds, sulfur compounds, furans, pyrazines, pyridines, amines and other volatile compounds comprise a broad range of volatile metabolites present in seaweeds [21]. There are significant differences in volatile compounds between seaweed species. The species, its geographical origin and processing parameters are known to influence its volatile profile [22].

The main aim of this review is to provide insights into the volatile analyses of six commercially important brown and red seaweed species used in the food industry. The most widely harvested, due to their abundance and accessibility, are the brown *Laminaria* spp. and the red *Palmaria palmata* seaweeds. This review focuses on the volatile fraction of edible seaweeds belonging to four species (*Himanthalia elongata*, *Laminaria* spp. including *Laminaria ochroleuca*, and *Undaria pinnatifida*) of brown seaweed, and two species (*Porphyra umbilicalis* and *Palmaria palmata*) of red seaweed due to their presence in Irish waters [23,24]. A data search using the major databases (PubMed, ScienceDirect, Springerlink, ResearchGate) with the keywords ("macroalgae", "seaweeds", "volatile compounds", "brown kelp", "*Himanthalia elongata*", "*Laminaria japonica*", "*Laminaria ochroleuca*", "*Palmaria palmata*", "*Porphyra umbilicalis*", "*Undaria pinnatifida*", "Winged kelp") highlighted that there is a lack of information on the volatile compounds for these seaweed species and therefore this review is timely as it provides a relevant summary of up to date information.

2. Sensory properties of seaweed volatile compounds

Volatile compounds perceived simultaneously define the odor of a food product [25]. Over 10,000 volatile compounds have been detected in foods, but only a few hundred (5–10%) have any significance as odor compounds [26]. Key odor compounds have odor threshold values in a very broad range (mg/l – pg/l) and two key odor features, odor threshold and compound concentration, must be taken into account when discussing the odor impact of compounds in foods [26]. The odor activity value (OAV) estimates the importance of a flavor compound in a food, based on the ratio of its concentration to its odor threshold concentration in that food. Only compounds which exceed the threshold level in a food will impact on flavor and thus sensory perception [25]. Conceptually, the larger the OAV, the more likely that compound will contribute to the overall odor of a complex odor mixture [27].Compounds with extremely low odor thresholds, even when present in very low concentrations, can be important odorants [26]. Gas chromatography-olfactometry (GC-O) is the main approach used to study the impact of volatile compounds on sensory perception.

Some analytes with sensory significance present in a food are at low concentrations, therefore they require isolation and concentration from the food prior to analysis [28]. The presence of trace quantities of odor components complicates the extraction/concentration process, to the extent that methods based on volatility, solubility and the use of organic solvents will also extract water, lipids, proteins and carbohydrates, respectively that may necessitate further processing prior to analysis by GC–MS. Odor isolation is difficult because volatiles have a very low molecular mass (< 300 Da) and belong to a large number of different chemical classes [28]. In relation to the different volatile chemical classes identified in these seaweeds, the most abundant were, in decreasing order, hydrocarbons, ketones, aldehydes, alcohols, esters, halogenated compounds, carboxylic acids, furans, phenols, sulfur compounds, pyrazines, pyridines, amines and some miscellaneous compounds.

Terpenes are potentially important odor compounds, where sesquiterpene hydrocarbons have lower impact than monoterpenes due to their higher detection thresholds [29]. Hydrocarbons are not always relevant to the aroma because of their high odor detection thresholds, but compounds with high odor thresholds can also play an essential role in food aroma when present at high concentrations [30]. Ketones are potentially important odor compounds due to their abundance and odor activity. Ketones, such as β-ionone and 6-methyl-5-hepten-2-one, are potent odorant in seafood contributing to the aroma composition of seaweeds [31]. The chain length of aldehydes mostly affects odor thresholds and odor properties. Aldehydes with low molecular weight (< 150 Da) tend to be associated with unpleasant odors, and those with higher molecular weights tend to have sweet, fruity odors [26]. Aldehydes are important aroma compounds because of their low odor threshold values [32]. Carbonyl compounds and aldehydes are responsible for various odorant notes such as the nutty alcoholic odor of acetaldehyde [33] and the almond odor of benzaldehyde [29]. Branched-chain alcohols are known to have low odor threshold values, therefore may significantly contribute to odor [32]. As the ability of alcohols to dissolve in water decreases the odor threshold increases. Therefore unsaturated alcohols have a greater impact on food flavor than saturated alcohols [26]. Halogenated compounds are mainly isolated from red and brown seaweeds [2]. Halogenated compounds substituted with a halogen moiety at the ortho position interact strongly with human odor receptors, resulting in high odor activities [34]. Aryl halides are fairly pleasant smelling liquids, but arylmethyl (benzylic) halides are irritating to nasal passages [35]. Acids may increase and enhance food characteristic odor profiles. The strength of odor decreases with increasing acid chain length. Simple acids, with < 6 carbon atoms, have high odor thresholds, while long chain acids, with 12 or more carbon atoms, are odorless. Unsaturated acids generally have sharper and stronger odors than saturated ones [26]. Esters present mainly fruity notes (e.g. apple odor of butyl acetate) and unsaturated esters have lower thresholds than saturated ones [29]. Furans consist of a family of important chemicals in the formation of crab flavor [36]. Lipoxygenase-catalysed oxidation of linoleic acid can produce 2-pentyl furan which has been associated with 'earthy', 'beany' odor notes [37]. Sulfur compounds are present in very low amounts and possess very low odor thresholds. They are present mainly in brassica vegetables as, for example, dimethyl sulfide responsible for cabbage odor [26]. Many pyrazines have intensive odors and very low odor thresholds. Therefore, these compounds contribute to many odors and flavors [38]. Heterocycles, in the pyridines group, are the main compounds of caramel odor [39]. Amines have a strong ammoniac-like odor. Amines are formed from Strecker degradation products, and their odors depend on the pH value [29]. Phenolic compounds contribute to the flavor of many foods [40].

The physicochemical properties of these compounds play a major role in sensory perception. Odor thresholds for particular compounds published in the literature can vary extensively and thus it is difficult to estimate an average odor threshold value based on literature data alone [26] (Table 1).

3. Key volatiles identified in commercially important seaweed species

3.1. Extraction and analytical methods

The major volatile components identified in commercially important seaweeds that likely have an impact on sensory perception are briefly summarized. The extraction/concentration conditions and polarity of the GC columns and methods of detection varied between studies [10,12,17,20,41,42].

López-Pérez et al. [22] used HS-SPME GC–MS with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber to identify volatiles in dried samples of five of the six seaweeds reviewed in this study (*H. elongata*, *U. pinnatifida*, *L. ochroleuca*, *P. umbilicalis* and *P. palmata*). These authors optimized the extraction/concentration conditions (equilibration time, extraction time and temperature and sample size to headspace volume ratio) and were able to identify a large number of volatiles compounds. They used a polyethylene glycol highly polar column and a single quadrupole MS over the mass range of 35 to 300 *m/z*.

Table 1
Volatile compounds found in four brown and two red species of edible seaweed.

Compound	*Himanthalia elongata*	*Laminaria* spp.		*Undaria pinnatifida*	*Palmaria palmata*	*Porphyra umbilicalis*	Aroma threshold
		Laminaria spp.	*Laminaria ochroleuca*				
Hydrocarbons							
Butane[c]		✓		✓			0.421–5048 ppm[g]
Hexane[c]		✓		✓			30–248 ppm[g]
Octane[a]	✓		✓	✓	✓	✓	0.66–235 ppm[g]
Nonane[a]	✓		✓	✓	✓	✓	2.3–21 ppm[g]
Undecane[e]				✓			
Dodecane[e]				✓			
Tetradecane[e]				✓			
Pentadecane[a,e]	✓		✓	✓	✓	✓	
Hexadecane[e]				✓			
Heptadecane[a,e]	✓			✓	✓	✓	
Octadecane[c]				✓			
6-Methylpentadecane[e]				✓			
2-Methylheptane[a]	✓		✓	✓	✓	✓	
4-Methylheptane[a]	✓		✓	✓	✓	✓	
4-Methyloctane[a]	✓		✓	✓	✓	✓	
2-Methylnonane[a]	✓		✓	✓	✓	✓	
4-Methyldecane[a]	✓		✓	✓	✓	✓	
5-Methyldecane[a]	✓		✓	✓	✓	✓	
2-Methylundecane[a]	✓		✓	✓	✓	✓	
3-Methylundecane[a]	✓		✓	✓	✓	✓	
4-Ethyldecane[a]	✓		✓	✓	✓	✓	
2,4-Dimethylhexane[a]	✓		✓	✓	✓	✓	
2,4-Dimethylheptane[a]	✓		✓	✓	✓	✓	
2,7-Dimethyloctane[a]	✓			✓	✓	✓	
2,5-Dimethylnonane[a]	✓		✓	✓	✓		
3,7-Dimethyldecane[a]	✓		✓	✓	✓	✓	
2,4-Dimethylundecane[a]	✓		✓	✓	✓	✓	
2,6-Dimethylundecane[a]	✓		✓	✓	✓	✓	
4,4-Dimethylundecane[a]	✓		✓	✓	✓	✓	
4,8-Dimethylundecane[a]	✓		✓	✓	✓	✓	
4,6-Dimethyldodecane[a]	✓			✓	✓	✓	
2,4,6-Trimethyloctane[a]	✓			✓	✓		
2,6,10-Trimethyl-tetradecane[e]				✓			
Cyclopentane, iodo[c]		✓					
Cyclododecane[b,c]	✓			✓			
Dicloromethane[c]		✓		✓			
Trichloromethane[c]		✓					
(E)-2-Octene[a]	✓		✓	✓	✓	✓	
2,4-Dimethyl-1-heptene[a]	✓		✓	✓	✓	✓	
1,4-Octadiene[a]	✓		✓	✓	✓	✓	
1,3-(*E*)-5-(*Z*)-Octatriene[f]					✓		
1,3-Cyclooctadiene[a]	✓		✓	✓	✓	✓	
Trimethyl-1,3-cyclopentadiene[f]					✓		
1,5-Heptadien-3-yne[e]				✓			
2-Methyl-1-hexen-3-yne[e]				✓			
3-Ethyl-2-methyl-1,3-hexadiene[a]	✓		✓	✓	✓	✓	
3-Ethylidene-1-methylcyclopentene[f]					✓		
2-(Chloromethyl)-1-butene[c]		✓					
1,3-Dimethylbenzene[a]	✓			✓	✓	✓	
1,2,3-Trimethylbenzene[a]	✓			✓	✓	✓	
1,2,4-Trimethylbenzene[f]					✓		
Ketones							
2-Propanone[a]	✓		✓	✓	✓	✓	40–476 ppm[h,j]
2-Butanone[a]	✓		✓	✓	✓	✓	n/a[h]
2-Pentanone[a]	✓		✓	✓	✓	✓	70 ppb[h,j]
3-Hexanone[a]	✓		✓	✓	✓	✓	41–81 ppb[h,j]
2-Heptanone[a]	✓		✓	✓	✓	✓	1 ppb-1.33 ppm[h,j] 2.66–3.73 ppm[h,k]
2-Octanone[a]	✓		✓	✓	✓	✓	41–62 ppb[h,j]
3-Octanone[d]		✓			✓		21–50 ppb[h,j]
1-Penten-3-one[c]				✓			1–13 ppb[h,j]
3-Penten-2-one[a]	✓		✓	✓	✓	✓	1.5 ppb[h,j]
1-Octen-3-one[a]	✓			✓	✓	✓	0.05–4 ppb[h,j]
3-Octen-2-one[a]	✓			✓	✓	✓	n/a[h]
2,3-Butanedione[a]	✓		✓	✓	✓	✓	1–8.6 ppb[i]
2,5-Octadione[a]	✓		✓	✓	✓	✓	
(Z,E)-3,5-Octadien-2-one[a]	✓		✓	✓	✓	✓	
(E,E)-3,5-Octadien-2-one[a,e]	✓		✓	✓	✓	✓	0.15 ppm[h,j]
5,9-Undecadien-2-one, 6, 10-dimethyl-, (z)[e]				✓			60 ppb–6.4 ppm[h,j]
3,5-Dimethyl-2,7-octadione[a]	✓		✓	✓	✓	✓	

(*continued on next page*)

Table 1 (*continued*)

Compound	*Himanthalia elongata*	*Laminaria* spp.		*Undaria pinnatifida*	*Palmaria palmata*	*Porphyra umbilicalis*	Aroma threshold
		Laminaria spp.	*Laminaria ochroleuca*				
1,3-Cyclohexanedione,2-(2-propenyl)-[e]				✓			
4,4-Dimethyl-2-cyclohexen-1-one[e]				✓			
2(1H)-naphthalenone, octahydro-8a-methyl-, trans-[e]				✓			
6-Methyl-3,5-heptadien-2-one[a]	✓		✓	✓	✓	✓	375 ppb[h,j]
6-Methyl-5-hepten-2-one[a]	✓		✓	✓	✓	✓	50 ppb[h,j]
1-Hydroxy-2-propanone[a]	✓			✓	✓	✓	
3-Hydroxy-2-butanone[a]	✓		✓	✓	✓		
2,2,6-Trimethylcyclohexanone[a,e]	✓			✓	✓	✓	100 ppb[h,j] 310 ppb[h,k]
3,5,5-Trimethyl-2-cyclohexen-1-one[a]	✓		✓	✓	✓	✓	
2-Propyl-2-cyclohexenone[a]				✓	✓	✓	
γ-Valerolactone[a]	✓		✓	✓	✓	✓	n/a[h]
γ-Butyrolactone[a]	✓		✓	✓	✓	✓	20–50 ppm[h,j]
1-Phenylethanone[a]	✓		✓	✓	✓	✓	170 ppb[h,j] 2.9 ppm[h,k]
γ-Hexalactone[a]	✓		✓	✓	✓	✓	1.6 ppm[h,j]
5-Ethyl-2(5H)-furanone[a]	✓		✓	✓	✓	✓	
Isophorone[e]				✓			0.20 ppm[h,j]
4-Ketoisophorone[a]	✓		✓	✓	✓	✓	n/a[h]
α-Ionone[a]				✓	✓	✓	0.6–10 ppb[h,j]
β-Ionone[a,e]	✓		✓	✓	✓	✓	0.007–205 ppb[h,j]
Trans-β-ionone[a]	✓		✓	✓	✓	✓	
7,8-Epoxy-α-ionone[a]	✓				✓	✓	
1-(4-Acetylphenyl)-ethanone[a]	✓		✓	✓	✓	✓	
Methylethylmaleimide[a]			✓	✓	✓	✓	
Dihydroactinidiolide[a]	✓		✓	✓	✓	✓	
Aldehydes							
Ethanal[a]	✓		✓	✓	✓	✓	0.7–200 ppb[h,j] 27–380 ppb[h,k]
Propanal[a]	✓		✓	✓	✓	✓	9.5–35 ppb[h,j]
Butanal[a,c]	✓	✓	✓	✓	✓	✓	19–37 ppb[h,j] 11–27 ppb[h,k]
Pentanal[a,d]	✓	✓	✓	✓	✓	✓	12–100 ppb[h,j]
Hexanal[a,c,d,e]	✓	✓	✓	✓	✓	✓	4.1–22.8 ppb[h,j] 400 ppb[h,k]
Heptanal[a,d,e]	✓	✓	✓	✓	✓	✓	3–60 ppb[h,j]
Octanal[a,c,e]	✓	✓	✓	✓	✓	✓	1.4–6.4 ppb[h,j]
Nonanal[a,c,d,e]	✓	✓	✓	✓	✓	✓	1–8 ppb[h,j]
Decanal[c]		✓		✓		✓	0.1-6 ppb[h,j] 9 ppb[h,k]
Undecanal[c]		✓					0.4–100 ppb[h,j]
Dodecanal[c]		✓					0.5–1.5 ppb[h,j]
(E)-2-Butenal[a]	✓		✓	✓	✓	✓	525 ppb[i,j]
(E)-2-Pentenal[a,c,e]		✓	✓	✓	✓	✓	1.5 ppb[h,j]
(E)-2-Hexenal[a,d,e]	✓	✓	✓	✓	✓	✓	17 ppb–10 ppm[i,j]
(E)-2-Heptenal[a,e]	✓	✓	✓	✓	✓	✓	13–51 ppb[j]
(Z)-4-Heptenal[a]	✓		✓	✓	✓	✓	0.4 ppb[h,j]
(E)-2-Octenal[a,d,e]	✓	✓	✓	✓	✓	✓	3–4 ppb[h,j]
(E)-2-Nonenal[d,e]		✓		✓			0.1 ppb[h,j]
(E)-2-Decenal[d,e]		✓		✓			1 ppb[h,j]
(E,E)-2,4-Heptadienal[a,c]	✓		✓	✓	✓	✓	n/a[h]
(Z,E)-2,4-Heptadienal[a]	✓		✓	✓	✓	✓	3.6 ppm[i,j]
(E,E)-2,6-Nonadienal[a]	✓		✓	✓	✓	✓	0.09 ppb[h,j]
(E,Z)-2,6-Nonadienal[e]				✓			
(E,E)-2,4-Decadienal[e]				✓			0.07–10 ppb[h,j]
(3Z)-3-Hexenal[c]				✓			0.25 ppb[h,j]
5-Hexenal[c]		✓					
2-Methylbutanal[a]			✓	✓	✓	✓	0.15–140 ppb[i,j]
3-Methylbutanal[a,d]	✓	✓	✓	✓	✓	✓	12 ppb[i,j]
(E)-2-Methyl-2-butenal[a]			✓	✓	✓	✓	500 ppb[h,j]
2-Methyl-2-propenal[a]					✓	✓	
2-Methyl-2-pentenal[a]				✓	✓	✓	290 ppb[h,k]
2,2-Dimethylpropanal[f]					✓		
Benzaldehyde[a]	✓		✓	✓	✓	✓	100 ppb − 4.6 ppm[h,j] 330 ppb–4.1 ppm[h,k]
4-Ethylbenzaldehyde[a]	✓		✓	✓	✓	✓	13 ppb[h,j]; 40 ppb[h,k]
4-Propylbenzaldehyde[a]	✓		✓	✓	✓	✓	
β-Cyclocitral[a,e]	✓		✓	✓	✓	✓	
Safranal[a]	✓		✓	✓	✓	✓	
Alcohols							

(*continued on next page*)

Table 1 (*continued*)

Compound	*Himanthalia elongata*	*Laminaria* spp.		*Undaria pinnatifida*	*Palmaria palmata*	*Porphyra umbilicalis*	Aroma threshold
		Laminaria spp.	*Laminaria ochroleuca*				
Ethanol[c]		✓		✓			8–900 ppb[h,j]
1-Propanol[c]		✓		✓	✓	✓	3.2–7500 ppm[i]
1-Butanol[d]		✓					500 ppb–509 ppm[h,j]
1-Pentanol[a]	✓		✓	✓			32 mg/L liquid[i]
3-Pentanol[a]	✓		✓	✓	✓	✓	
1-Hexanol[a,c]	✓	✓	✓	✓	✓	✓	0.01–5.2 ppm[i]
1-Octanol[a]	✓		✓	✓	✓	✓	42–480 ppb[h,j]
3-Decanol[c]				✓			79–410 ppb[h,j]
1-Penten-3-ol[a,c,d]	✓	✓	✓	✓	✓	✓	400 ppb[h,j]
(E)-2-Penten-1-ol[a]	✓		✓	✓	✓	✓	
(Z)-2-Penten-1-ol[a,c]	✓	✓	✓	✓	✓	✓	
3-Hexen-1-ol[c]		✓		✓			70 ppb[h,j]
5-Hexen-1-ol[c]				✓			
(2E)-2-Hexen-1-ol[c]				✓			
1-Octen-3-ol[a,c,d]	✓	✓	✓	✓	✓	✓	14 ppb[h,j]; 25 ppb[h,k]
2-Octen-1-ol[a]	✓		✓	✓	✓	✓	40–840 ppb[h,j] 100 ppb[h,k]
(E)-2-Octen-1-ol[d]		✓					n/a[h]
(E)-2-Nonen-1-ol[d]		✓					130 ppb[h,j]
2,3-Butanediol[a]				✓			
(Z)-1,5-Octadien-3-ol[a]	✓		✓	✓	✓	✓	
1,7-Octadien-3-ol[a]	✓		✓	✓			
3,5-Octadien-2-ol[a]	✓		✓	✓	✓		
3-Methylbutanol[f]					✓		250 ppb–4.1 ppm[h,j]
2-Methyl-3-pentanol[a]	✓		✓	✓	✓		
(E)-2-Ethyl-1-hexanol[a]	✓		✓	✓	✓		n/a[h]
(Z)-2-Ethyl-1-hexanol[a]	✓						n/a[h]
2-(2-Methoxypropoxy)-1-propanol[a]	✓		✓	✓	✓	✓	
1-(2-Methoxypropoxy)-2-propanol[a]	✓		✓	✓	✓	✓	
1-(2-Methoxy-1-methylethoxy)-2-propanol[a]	✓		✓	✓	✓	✓	
1-Ethoxy-2-propanol[a]	✓		✓	✓	✓	✓	
Cyclooctanol[a]	✓			✓	✓	✓	
1,2-Dimethyl-cyclohexanol[a]	✓		✓	✓	✓	✓	
2,4-Dimethyl-cyclohexanol[e]				✓			
4,4-Dimethyl-cyclohex-2-en-1-ol[c]				✓			
Benzenemethanol[a]	✓		✓	✓	✓	✓	1.2–1000 ppb – 10–1000 ppm[h,j]
Halogenated compounds							
Iodo-methane[a]			✓		✓		
Iodo-ethane[a]			✓		✓		
2-Iodo-propane[a,c]		✓	✓	✓	✓		
1-Iodo-pentane[a,c]	✓		✓	✓	✓		
1-Iodo-octane[d]		✓	✓				
2-Fluoroprop-1-ene[f]					✓		
Chlorobenzene[f]					✓		0.21 ppm[i]
Dichloromethane[f]					✓		210–214 ppm[i]
Tribromomethane[d]		✓			✓		4.8 × 10^8 molecules/cc[i]
Trichloromethane[d]		✓			✓		
5-Chloro-1-pentene[a]			✓				
Carboxylic acids							
Ethanoic acid[a]	✓		✓	✓	✓	✓	10–522 ppm[j] 60 ppm[h,k]
Butanoic acid[a]	✓		✓	✓	✓	✓	240 ppb–4.8 ppm[h,j]
Hexanoic acid[a]	✓		✓	✓	✓	✓	93 ppb–10 ppm[h,j]
Heptanoic acid[a]	✓		✓	✓	✓	✓	640 ppb–10.4 ppm[h,j]
Octanoic acid[a]	✓		✓	✓	✓	✓	910 ppb–19 ppm[h,j]
2-Methylpropanoic acid[a]	✓		✓	✓		✓	10 ppb–9.5 ppm[h,j]
3-Methylbutanoic acid[a]	✓		✓	✓	✓	✓	
Esters							
Acetic acid ethyl ester[c]				✓			5 ppb–5 ppm[h,j]
Lactic acid ethyl ester[a]				✓			50–250 ppm[h,j]
Octyl benzoate[e]				✓			
Propanoic acid, 2-methyl-0.1-(1,1-dimethylethyl)-2-methyl-1,3,-propanediyl ester[e]				✓			
Hexanedioic acid, bis(2-methylpropyl) ester[e]				✓			n/a[h]
Dodecanoic acid, 1-methyl ester (isopropyl laurate)[e]				✓			n/a[h]
Pentanedioic acid, dibutylester[c]		✓		✓			
Phthalic acid, diisobutylester[e]				✓			
1,2,3-Propanetriol[a] triethanoate				✓			n/a[h]

(*continued on next page*)

Table 1 (*continued*)

Compound	*Himanthalia elongata*	*Laminaria* spp.		*Undaria pinnatifida*	*Palmaria palmata*	*Porphyra umbilicalis*	Aroma threshold
		Laminaria spp.	*Laminaria ochroleuca*				
1,2-Benzenedicarboxylic acid diethyl ester[a]	✓			✓	✓	✓	
Furans							
2-Ethylfuran[a,c,e]	✓		✓	✓	✓	✓	n/a[h]
2-Methylfuran[a]	✓		✓	✓	✓	✓	27 ppm[i]
2-Pentylfuran[a]	✓		✓	✓	✓	✓	6 ppb[h,j]
cis-2-(2-Pentenyl)furan[a]	✓		✓	✓	✓	✓	
Sulfur compounds							
Dimethyl sulfoxide[a]	✓		✓	✓	✓	✓	
Dimethyl sulfide[a]	✓		✓	✓	✓	✓	0.3–10 ppb[h,j]
Phenols							
Phenol,2,4-bis (1,1-dimethylethyl)-[b]	✓						
Phenol,2,6-bis (1,1-dimethylethyl)-4-methyl[b]	✓						
Amines							
N,*N*-Dimethyl-methanamine[a]	✓		✓		✓	✓	0.3–0.8 ppb[h,j] 500 ppb[h,k]
Pyrazines							
2,6-Dimethylpyrazine[a]			✓	✓			400–1500 ppb[h,j]
2,3,5,6-Tetramethylpyrazine[a]					✓		1–10 ppm[h,j]
Pyridines							
2-Ethylpyridine[a]				✓	✓		4–22 ppm[h,j]
Miscellaneous							
1-Acetyl-2,2-dimethylcyclobutane[a]			✓				
1,2-Benzisothiazole[e]				✓			
1,3-Cyclopentadiene, 5,5-dimethyl-2-ethyl-[e]				✓			
1-Methoxy-4-(1′-methylethyl)cyclohexa-1,4-diene[a]	✓		✓	✓	✓		
2,5-Dimethylhexane-2,5-dihydroperoxide[e]				✓			
2,7-Epoxy-megastigma-4,8-diene[a]	✓		✓	✓			
Stigmasta-5,24(28)dien-3-ol (Fucosterol)[a]	✓						

✓ Identified/Present in the seaweed.

Brown species: *Himanthalia elongata*, *Laminaria* spp., *Undaria pinnatifida*, *Laminaria ochroleuca*. Red species: *Palmaria palmata*, *Porphyra umbilicalis*.

[a] [22].
[b] [18].
[c] [11].
[d] [42].
[e] [13].
[f] [41].
[g] [52], diluent was assumed to be air, unless specified otherwise in the paper.
[h] [53], representative values in distilled water unless otherwise designated.
[i] [54], representative values in water unless otherwise designated.
[j] Detection.
[k] Recognition.

Balbas et al. [13] also used HS-SPME with a DVB/CAR/PDMS fiber, but incubated 0.5 g of dried sample (*U. pinnatifida*) for 30 min at 35 °C in a 10 mL headspace vial with 2 mL deionized water. The column was a 5% 1,4-bis(dimethylsiloxy)phenylene/95% dimethyl polysiloxane low polarity column. The mass scan of the single quadrupole MS was from *m/z* 48 to *m/z* 400.

Ferraces-Cascais et al. [11] used a purge and trap (sorbent not provided) to extract volatiles from fresh samples of two types of macroalgae (*Laminaria* spp. and *U. pinnatifida*) by a 5% diphenylpolysiloxane and 95% dimethyl-polysiloxane low polarity column with a single quadrupole MS, scanning a mass range of *m/z* 35–400. Le Pape et al. [41] also used purge and trap (Tenax TA 80–100 mesh) at 25 °C; and a polyethylene glycol highly polar column and a single quadrupole MS over the mass range 33–300 *m/z*.

Takahashi et al. [42] used a capillary column (no specifications) to extract volatiles from *Laminaria* spp. Plaza et al. [18] used simultaneous distillation and extraction under reduced pressure and an accelerated solvent extractor with hexane, ethanol and water as solvents to extract volatiles from *H. elongata*. The GC column was a fused silica capillary column coated with a 0.25 μm layer of 94% methyl, 5% phenyl, 1% vinyl polysilicone (low polarity), coupled to a quadrupole MS using a mass interval ranging from 35 to 450 *m/z*. These authors also optimized extraction conditions in terms of solvents and temperature.

3.2. Volatile compounds of seaweed species

3.2.1. Brown seaweed species

3.2.1.1. Himanthalia elongata (sea spaghetti). *Himanthalia elongata* or 'sea spaghetti' belongs to the Phaeophyta or brown macroalgae [43]. This edible brown seaweed is commonly harvested along the European side of the Atlantic Ocean [23]. It has a history of safe use and acceptability in cooking and was previously used to add a beefy or nutty-like flavor to dishes [43]. Indeed, beef patty formulations containing *H. elongata* seaweed were improved in texture and mouthfeel preserving their sensory quality, and were of the most overall accepted [44].

López-Pérez et al. [22] found 34 hydrocarbons in *H. elongata*, but only 30 could be positively identified. The most abundant branched-

chain hydrocarbon was 4-ethyldecane and the most abundant linear saturated hydrocarbon was octane. Plaza et al. [18] only identified one hydrocarbon, cyclododecane, in *H. elongata*. López-Pérez et al. [22], identified 31 ketones; 2-propanone (acetone) predominated among linear saturated ketones, 3,5-octadien-2-one among linear unsaturated ketones and 3-hydroxy-2-butanone among branched-chain unsaturated ketones. *H. elongata* had the lowest concentration of volatile aldehydes of the seven seaweeds included in their study [22]. These same authors identified 22 aldehydes, with hexanal as the main linear saturated aldehyde, and (E)-2-hexenal as the main linear unsaturated aldehyde and benzaldehyde the main aromatic aldehyde. They also identified 22 volatile alcohols, with 1-(2-methoxypropoxy)-2-propanol the most abundant branched-chain alcohol, the most abundant linear unsaturated alcohols were 1-octen-3-ol and 1-penten-3-ol, with 1-pentanol as the most abundant linear saturated one. Only one halogenated compound (1-iodopentane), one ester (1,2-benzenedicarboxylic acid diethyl ester), and one amine (dimethylmethanamine) were detected in *H. elongata*. Seven carboxylic acids were also identified, the most abundant linear acid was ethanoic (acetic) acid, whereas the most abundant branched-chain acid was 3-methylbutanoate. Four furans were also detected; 2-methylfuran, cis-2-(2-pentenyl)furan, 2-ethylfuran and 2-pentyl-furan, with the latter two being the most predominant. Two sulfur compounds, dimethyl sulfide and dimethyl sulfoxide were also detected as were two dienes, 7-epoxy-megastigma-4,8-diene and 1-methoxy-4-(1′-methylethyl)cyclohexa-1,4-diene.

Plaza et al. [18] also identified two phenols; 2,4-bis-(1,1-dimethylethyl)- and phenol,2,6-bis-(1,1-dimethylethyl)-4-methyl; two fatty acids, tetradecanoic (myristic) and hexadecanoic (palmitic) acid; stigmasta-5,24(28)dien-3-ol (fucosterol) and cyclododecane in *H. elongata.* The volatile compounds identified in *H. elongata* can be found in Table 1.

3.2.1.2. Laminaria spp.. *Laminaria* spp. are members of brown algae, the most important seaweed cultured in the Pacific Ocean from the economical point of view [45]. The yearly output of this seaweed is approximately 15 million tons in dry weight [46]. At present, it is valued mainly as food and widely consumed in East Asia [47].

Ferraces-Casais et al. [11] identified 26 volatile compounds in *Laminaria* spp.; six were hydrocarbons, with butane the most abundant. The hydrocarbons constituted the second most abundant family of volatiles present. Alcohols were the most important class of volatile compounds identified (29% of compounds identified). Seven alcohols were identified with 1-penten-3-ol as the most predominant one. Takahashi et al. [42] also identified 1-penten-3-ol and 1-butanol in Kombu. Ten aldehydes were identified with hexanal the most abundant. Takahashi et al. [42] also identified pentanal, 2-(*E*)-hexenal, hexanal, heptanal, nonanal in Kombu. Pentanedioic acid, dibutylester was the only ester identified by Ferraces-Casais et al. [11]. Six halogenated compounds were also identified, among them 1-iodo-pentane and 2-iodo-propane. Takahashi et al. [42] found in addition iodopentane, trichloromethane and tribromomethane. The low amount of chemical classes identified is more likely due to the extraction method (purge and trap) employed in relation to the polarity of the compounds present. Takahashi et al. [42] studied volatile compounds of dried Kombu (*Laminaria* spp.) by SDE and DHE. The 49 volatiles of Kombu analyzed by DHE were almost the same as identified using SDE, except trichloromethane and tribromomethane were not detected by DHE, and 3-methylbutanal, (E)-2-octenal, (E)-2-decenal (aldehydes); (E)-2-octen-1-ol, (E)-2-nonen-1-ol (alcohols) and 1-iodo-octane (halogenated compound) were only present by DHE. Only 1-iodooctane had an odor from the 4 kinds of iodides detected. The volatile compounds identified in *Laminaria* spp. can be found in Table 1.

Laminaria ochroleuca belongs to brown algae (Filum Ochrophyta, Class Phaeophyceae of the family of Laminariaceae). *L. ochroleuca* specimens grow in subtidal areas (from Morocco to southern UK and in the Mediterranean), and are abundant in the Atlantic coast of Cadiz (Southern Spain) [51].

López-Pérez et al. [22] identified 128 volatile compounds in *L. ochroleuca*. The numbers varied from 30 hydrocarbons, to 26 aldehydes, 7 carboxylic acids, to none esters. Volatile ketones, alcohols and furans reached their lowest levels in *L. ochroleuca* compared to the other species studied. Twenty-seven ketones, 20 alcohols and 4 furans were identified. Total volatile halogenated compounds reached their highest in *L. ochroleuca*, which contained 6 halogenated compounds. The most abundant one was iodo-methane followed by 2- and 1-iodopentane. Other volatile compounds detected in *L. ochroleuca* were 1-acetyl-2,2-dimethylcyclobutane, 1-methoxy-4-(1′-methylethyl)cyclohexa-1,4-diene and 2,7-epoxy-megastigma-4,8-diene. A summary of the volatile compounds identified in *L. ochroleuca* can be found in Table 1.

3.2.1.3. Undaria pinnatifida (Wakame). *Undaria pinnatifida* or Wakame is a type of brown seaweed that normally grows in the seas of Japan, Korea and New Zealand, but is also commonly found along Western European coasts as an invasive species [48]. Its structure can be divided into the blade (lamina), midrib, sporophyll and root-like formations (haptera) [49]. Wakame is one of the most important global economic marine algae and has a long-standing tradition as food for human consumption [50].

López-Pérez et al. [22] identified 35 hydrocarbons in *U. pinnatifida*, where 4-ethyldecane, pentadecane and 2-methylnonane were the most abundant. The number of hydrocarbons in the volatile fraction of *U. pinnatifida* found in other studies varied from 10 alkanes in Balbas et al. [13] to two hydrocarbons in Ferraces-Casais et al. [11], however differences are likely related to the choice of extraction/concentration methods and operating conditions. Hexadecane and heptadecane [13] and butane [11] were the most abundant hydrocarbons.

López-Pérez et al. [22] also found 34 ketones; with (Z,E)-3,5-octadien-2-one, and 2-propanone and 2-butanone as predominant ones among linear unsaturated and linear saturated ketones, respectively. Balbas et al. [13] identified eight volatile ketones in *U. pinnatifida*, with both (E,E)-3,5-octadien-2-one and β-ionone the most abundant ones, whereas Ferraces-Casais et al. [11] only identified 1-penten-3-one.

Benzaldehyde was the major aromatic aldehyde, 2-hexenal the major linear unsaturated aldehyde and hexanal was by far the major linear saturated aldehyde [22]. Thirteen [13] and seven [11] aldehydes were observed in dried *U. pinnatifida*. Hexanal and 2-heptenal were the most abundant aldehydes found by Balbas et al. [13], while hexanal was the most abundant one in the study by Ferraces-Casais et al. [11].

López-Pérez et al. [22] identified 22 alcohols, the most abundant branched-chain alcohol was 1-(2-methoxypropoxy)-2-propanol, while 1-octen-3-ol and 1-penten-3-ol predominated among linear unsaturated alcohols. There was also a high concentration of (Z)-1,5-octadien-3-ol. Ferraces-Casais et al. [11] reported nine alcohols while Balbas et al. [13] only identified three. The main alcohols were 1-penten-3-ol [11] and 3-decanol [13].

U. pinnatifida also contained six halogenated compounds and seven carboxylic acids in the López-Pérez et al. [22] study, with ethanoic (acetic) acid the most plentiful. *U. pinnatifida* also had an 1-iodo-pentane as the only halogenated compound [11]. Only Balbas et al. [13] perceived carboxylic acids, 2-methylpentanoic and 9-hexadecenoic acids in *U. pinnatifida*.

1,2-Benzenedicarboxylic acid diethyl ester appeared in greater amounts in all seaweed samples than *U. pinnatifida*, while lactic acid ethyl ester and 1,2,3-propanetriol triethanoate were only identified in *U. pinnatifida* [22]. Ferraces-Casais et al. [11] found two esters in *U. pinnatifida*, with the most abundant dibutyl ester of pentanedioic acid, while Balbas et al. 2015 [13] found five, with hexanedioic acid, bis(2-methylpropyl) as the predominant ester.

López-Pérez et al. [22] identified four furans, with the greatest concentration of 2-ethyl-furan and 2-pentyl-furan. The volatile fraction of *U. pinnatifida* in other studies also included 2-ethyl-furan [11,13].

A low abundance of sulfur compounds was evident in *U. pinnatifida*.

The two sulfur compounds identified were dimethyl sulfide and dimethyl sulfoxide [22]. One pyrazine, 2,6-dimethylpyrazine; and one pyridine, 2-ethylpyridine were also detected [22]. Two other volatile compounds were also detected; 2,7-epoxy-megastigma-4,8-diene and 1-methoxy-4-(1′-methylethyl)cyclohexa-1,4-diene [22]. 1,2-Benzisothiazole, 1,3-cyclopentadiene, 5,5-dimethyl-2-ethyl- and 2,5-dimethylhexane-2,5- were also detected by Balbas et al. [13] in *U. pinnatifida*. A summary of the volatile compounds identified in *U. pinnatifida* can be found in Table 1.

3.2.2. Red seaweeds species

3.2.2.1. Palmaria palmata (Dulse). *Palmaria palmata* (*Rhodymenia palmata*) is a red alga (Rhodophyta) in the family Palmariaceae. It grows on the northern coasts of Pacific and Atlantic oceans. It is commonly known as 'dulse' [55], and it has recently become popular as a foodstuff [56]. In Ireland especially and many parts of the British Isles, *P. palmata* is widely available and has a strong historical tradition as component of the local diet [57].

López-Pérez et al. [22] identified 31 hydrocarbons in *P. palmata.* Heptadecane was the most abundant linear saturated hydrocarbon and 3-methylundecane the second most abundant hydrocarbon. Le Pape et al. [41] identified four hydrocarbons, with 3-ethylidene-1-methyl-cyclopentene as the most abundant one.

Thirty two ketones were identified of which 2-propanone (acetone) and 2-butanone were the most abundant [22], while Le Pape et al. [41] identified only two ketones (2,3-pentanedione and 3-octanone).

P. palmata had the highest concentration of aldehydes in the study by Lopez-Perez et al. [22], with 28 aldehydes identified. Propanal was the major linear saturated aldehyde and (E)-2-pentenal the major linear unsaturated aldehyde and benzaldehyde the major aromatic aldehyde. Le Pape et al. [41] identified seven aldehydes with octanal and nonanal as the most predominant.

There was also a high concentration of volatile alcohols, corresponding to 20 different compounds [14]. The most abundant branched-chain alcohol was 1-(2- methoxypropoxy)-2-propanol, while 1-octen-3-ol and 1-penten-3-ol predominated among linear unsaturated alcohols and 1-pentanol among linear saturated alcohols [22]. Le Pape et al. [41] identified three alcohols (1-butanol, 1-penten-3-ol and 3-methylbutanol) in *P. palmata*.

P. palmata also contained three halogenated compounds, the most abundant was 1-iodopentane; six carboxylic acids, withethanoic (acetic) acid the most abundant and one ester (1,2-benzenedicarboxylic acid diethyl ester). *P. palmata* also contained high abundances of dimethyl sulfide and dimethyl sulfoxide and two pyrazines, 2,6-dimethylpyrazine and 2,3,5,6-tetramethylpyrazine were also identified. In dried *P. palmata* samples, other pyrazines; 2,5-diethylpyrazine and 3-ethyl-2,5-dimethylpyrazine were also reported [58]. The only pyridine and amine detected by López-Pérez et al. [22] were 2-ethylpyridine and dimethylmethanamine, respectively, and were the most abundant in *P. palmata* in comparison to the other aldehydes. Le Pape et al. [41] identified seven halogenated compounds, the most abundant were iodoethane, tribromomethane and chlorobenzene. Neither carboxylic acids nor esters, furans, sulfur compounds, pyrazines or any other volatile compounds were detected by Le Pape et al. [41] in *P. palmata* samples, but this could be due to the DHE extraction/concentration methodology used.

The volatile compounds identified in *P. palmata* can be found in Table 1.

3.2.2.2. Porphyra umbilicalis (Nori). *Porphyra umbilicalis* (commonly known as Nori) is a macrophytic red alga –Rhodophyta- that belongs to an ancient group of red algae, the Bangiophyceae. It is important ecologically and economically [59] and is harvested for human food, and thrives in the harsh conditions of the upper intertidal zone [60].

López-Pérez et al. [22] detected 32 hydrocarbons in *P. umbilicalis* which was the lowest number detected in the seven seaweeds evaluated, four of them were unidentified branched-chain hydrocarbons. The most abundant were 3-methylundecane, 4-methyldecane and 2-methylnonane. These authors identified a considerable number (34) of ketones in *P. umbilicalis*, with (E,E)-3,5-octadien-2-one as the most abundant. Twenty seven aldehydes were also detected, with both hexanal and pentanal as the most abundant. The most abundant alcohols were 1-octen-3-ol and 1,2-dimethylcyclohexanol. No halogenated compounds were detected. The authors identified seven carboxylic acids, with both ethanoic (acetic) and hexanoic acids the most abundant and also one ester (1,2-benzenedicarboxylic acid diethyl ester). These authors identified 4 furans in *P. umbilicalis* with 2-ethyl-furan and 2-pentyl-furan as the most abundant and only 2 pyrazines, 2,6-dimethylpyrazine and 2,3,5,6-tetramethylpyrazine. The volatile compounds identified in *P. umbilicalis* can be found in Table 1.

4. Discussion and future perspectives

Macroalgae are already incorporated as nutritional ingredients into food and drinks, but the search for the ideal macroalgae for a wide range of applications is still on-going. The addition of small amounts of seaweeds or their extracts to food products opens up new prospects for food processing, meat product formulations included. A major difficulty with seaweeds and similar plants is that biological and chemical characteristics may change during growth, in the period from harvest to consumption, and during processing. These changes pose challenges in identifying key character impact volatile(s) that may influence sensory perception in processed foods to which they have been added.

Specific knowledge gaps exist in these chosen macroalgae species regarding, the effect of different harvesting, storage and processing methods of seaweeds on volatile composition; the identification of key volatile compounds that impact sensory perception and the information on the application of seaweeds in processed foods from a sensory perspective.

Regarding storage, the quality of the product is determined by its stability during storage, among other factors (thickness, hardness, color and the absence of foreign materials) [13]. Ideally samples should be extracted and analyzed soon after collection because compounds may change even if frozen (e.g. under nitrogen gas) [61]. However, it is often necessary to store samples for subsequent analysis, due to common setbacks. In any case, analysis of a few representative samples should be made, whenever possible, soon after collection so qualitative and quantitative changes in chemical composition can be detected. When immediate analysis is impossible, samples should be stored in a manner that minimizes changes in metabolite composition. For this purpose, further research [61,62] is required.

Regarding the processing, the nature of many secondary metabolites suggests that some may be labile, necessitating the use of methodologies that do not alter the concentration or structure of compounds [61]. Some authors have studied the influence of processing methods on the volatile compounds of different seaweeds, and depending on the method of their preparation the volatile content may vary greatly [11,42]. However, deeper knowledge on this aspect is also limited and merits further investigation.

It is evident that the production of volatile organic compounds by seaweeds is closely related to the physiology of the species, habitats, maturity [4,63], geographical origin and environmental conditions [22]. Algae are vegetative organisms and must adapt to abiotic stresses (humidity, mineral composition and the sea temperature, light amount and intensity) during their life cycle [44,64]. Therefore, the different compositions and properties of the volatile organic compounds imply different growth conditions of the same species [12].

Furthermore, consumer acceptance of food is closely related to its sensory properties, of which flavor is of prime importance. Some volatile compounds have great impact on the aroma of seaweeds and are considered "character-impact compounds". The diversity of these volatiles compounds present in seaweeds produce different odors like

"seafood", "marine", "fish", "licorice", "spices", "fatty", "green" and "honey" [65].

Carboxylic acids are related to odor notes such as spices, honey and licorice, while esters are related to green odor. Notes like fish, marine, fatty and seafood odor are related to presence of amines and pyridines. When present in higher concentrations, compounds contribute to fruity notes in aroma characteristics of some seaweeds [66]. Another class of compounds commonly found in the aroma composition of seaweeds are the ketones, such as β-ionone and 6-methyl-5-hepten-2-one, both potent odorants in seafood. The latter ketone results in compounds having more pleasant odors in seaweed and other foods, such as tomato [65]. Furans are evident in some species of seaweed and are important compounds in the formation of crab flavor, as highlighted by Gu et al. study [36]. Character-impact compounds also include halogenated compounds containing bromine (e.g. dibromoethane, dibromochloromethane, bromodichloromethane and chloroiodomethane) and iodine (e.g. iodoethane, iodopentane) and isoprene (2-methyl-1-3-butadiene), which contribute to the characteristic aroma of the seaweeds, with aromas like marine, crustacean and green [67]; and sweet notes [68], especially in red seaweeds [69].

The aroma diversity of seaweeds is a result of the variety and concentration range of the aroma compounds. Some of these aroma characteristics may be desirable in some applications but unwanted in others. Therefore, further studies to elucidate which edible seaweeds are more suitable in food and feed formulations are necessary. The detailed identification of volatile compounds is very important to elucidate their aroma properties of the final product [19].

5. Conclusion

The production of volatile organic compounds by plants/algae is well known. However, little research has been undertaken on the volatile compounds present in commercially important brown seaweeds (*Himanthalia elongata*, *Laminaria* spp. *Undaria pinnatifida*, and *Laminaria ochroleuca*) or red seaweeds (*Porphyra umbilicalis* and *Palmaria palmata*).

More than 200 different volatile compounds, belonging to 13 or more chemical groups, were present in the six seaweed species reviewed. There was only a limited amount of information available on *Laminaria* spp.

The major volatile compounds found in seaweeds, in decreasing order, were hydrocarbons, ketones, aldehydes, alcohols, halogen or sulfur-containing compounds, acids, esters, furans, and phenols. Volatile compounds reported in previous works depended, mostly, on the extraction methodology and GC–MS conditions. The impact of volatile compounds on sensory perception is dependent upon abundance and odor threshold.

Extended work needs to be done related to the volatile compounds of seaweeds in connection with their sensory characteristics. Further work is aimed at evaluating the aroma characteristics of seaweeds with the objective of evaluating the influence of flavor and odor notes on acceptability of new seaweed-containing products.

CRediT authorship contribution statement

Elena Garicano Vilar: Conceptualization, Writing - original draft, Writing - review & editing.**Maurice G. O'Sullivan:**Supervision, Writing - review & editing.**Joseph P. Kerry:**Supervision, Writing - review & editing.**Kieran N. Kilcawley:**Supervision, Writing - review & editing.

Declaration of competing interest

None.

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A chemometric approach to characterize the aroma of selected brown and red edible seaweeds / extracts

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Abstract

BACKGROUND: Information pertaining to the aromatic profile of seaweeds and seaweed extracts can provide evidence regarding their potential suitability as ingredients in processed foods. To date only limited material has been available on the volatile profiles of some seaweed species. Others in this study have not previously been described. The volatile profiles of dried brown (*Himanthalia elongata, Undaria pinnatifida, Alaria esculenta*) and red (*Porphyra umbilicalis, Palmaria palmata*) seaweeds, and a brown seaweed extract (fucoxanthin) from *Laminaria japonica* were investigated using a chemometric approach to collate data from volatile gas chromatography – mass spectrometry (GC–MS), direct sensory aroma evaluation, and gas-chromatography – olfactometry (GC-O) to obtain a better understanding of their volatile profile and sensory perception.

RESULTS: More than 100 volatile compounds were identified by static headspace solid phase micro-extraction (HS-SPME) and thermal desorption gas chromatography – mass spectrometry (TD GC–MS). Brown seaweeds were characterized by 'grassy/herbal/floral', 'fruity', and 'fatty' aromas, red seaweeds by 'green/vegetable', 'mushroom/earthy' and 'sweet/buttery' aromas, and the fucoxanthin extract by 'rancid' and 'nutty' aromas with an overall lower intensity. Heptanal appeared to be a major odor-active compound in all samples. Other volatiles were more characteristic of each individual seaweed: hexanal, (*E,Z*)-2,6-nonadienal and 2-pentylfuran for *H. elongata;* ethyl butanoate and 2,3-butanedione for *U. pinnatifida;* 6-dimethylpyrazine, (*E,Z*)-2,6-nonadienal and sulactone for *P. palmata;* 1-octen-3-ol for *P. umbilicalis,* heptanone for *A. esculenta,* and 2-furanmethanol for fucoxanthin.

Conclusion: Brown and red seaweeds had distinct sensory properties with individual seaweeds having differing volatiles and odorants. This study provides additional information that can contribute to the development of products incorporating dried seaweeds / extracts that are more acceptable to the consumer.
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Supporting information may be found in the online version of this article.

Keywords: seaweeds; volatile compounds; aroma; GC–MS; GC-O

INTRODUCTION

Volatile compounds are fundamental for the aroma and odor perception of seaweed.[1] The volatile compounds of brown and red seaweed have been investigated previously.[2–4] However, little information is available on species such as *Himanthalia elongata*, *Undaria pinnatifida*, *Alaria esculenta*, *Laminaria japonica*, *Porphyra umbilicalis*, and *Palmaria palmata*, which are found in abundance around the coastlines of Western Europe (see www.algaebase.org).[5–9] From an extensive review of the scientific literature, there appears to be no publications pertaining to the volatile profiles and odor characteristics of the seaweeds *A. esculenta* and *L. japonica*.

Gas chromatography–mass spectrometry (GC–MS) is one of the most powerful analytical methods used in flavor chemistry. Complex mixtures are separated by GC, and the components are then identified by MS.[10] Many methods exist for the extraction and preconcentration of analytes in solid samples.[11] However, no single extraction method is perfect, as each has a degree of bias, mainly due to issues relating to polarity, molecular weight, or vapor pressure.[12] A definite movement towards automated approaches has occurred due to ease of use, time efficiency, and general effectiveness.[12] To date, the extraction of volatile compounds from seaweeds has been carried out by dynamic headspace extraction

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(purge and trap),[13] distillation-solvent extraction[14] and static headspace solid phase micro-extraction (HS-SPME).[11] In this study, two automated extraction techniques were evaluated: (i) HS-SPME a static headspace technique, widely used for the identification of volatile organic carbons (VOCs) because of its ease of use and wide range of fibers available to target different chemical classes; and (ii) thermal desorption (TD), a dynamic technique that has a much larger sorbent-phase capacity with an enrichment capability.

Gas chromatography olfactometry (GC-O) uses human assessors in parallel with a detector, such as flame ionization or a mass spectrometric (MS) detector, to obtain sensory and chemical responses for aroma compounds, which enable the identification of the key odorants that exert the greatest impact on sensory perception.[15] Gas chromatography olfactometry has been extensively applied for the characterization of aroma impact compounds in a variety of matrices, mainly in food-related fields like coffee,[16] tea,[17] meat,[18] and others.[19] Nevertheless, very few studies[20,21] have utilized GC-O to evaluate seaweeds and / or seaweed extracts. The volatiles in seaweeds produce a range of different odors that could be categorized as 'marine' or 'seafood'. Other odors such as 'fish', 'fatty', 'honey', 'green', 'floral', and 'spicy' have also been detected in various green, red, and brown seaweeds.[1,22] In this study, HS-SPME GC-O was employed to elucidate the key aroma compounds in five dried edible seaweed species and in an edible seaweed powder extract. An aroma assessment was also undertaken to provide a more expansive profile, because a potential weakness of GC-O is that assessors can only sniff individual or co-eluted volatiles and may miss any aromas generated from the combination of volatiles and possibly aspects relating to the composition of the product.

The primary objective of this study was to use a chemometric approach to investigate the aroma of five dried edible seaweed species: four brown (one fucoxanthin, in the form of a powder extract), and two red species, to determine their main odor active properties.

MATERIAL AND METHODS

Seaweed material

The edible seaweeds used in this study were four brown seaweed species (*H. elongata*, *U. pinnatifida*, *A. esculenta*, and *L. japonica*) and two red species (*P. umbilicalis* and *P. palmata*). *Himanthalia elongata*, *P. umbilicalis*, *P. palmata*, and *A. esculenta* were supplied by Wild Irish Seaweed Ltd (Quilty, Co. Clare, Ireland). *Undaria pinnatifida* was bought from Algamar (Pazos de Borben, Pontevedra, Spain) and *L. japonica* fucoxanthin powder extract was shipped by Nutra Green Biotechnology Co. Ltd (Shanghai, China). Seaweeds were harvested along the west coast of Ireland, the north coast of Spain, or in China. The seaweeds from Wild Irish Seaweed were air dried and dehumidified (Caherush Point, Spanish Point, Co. Clare, Ireland) and Algamar seaweeds were dried at low temperature (<42 °C) at Polígono de Amoedo (Pazos de Borbén, Pontevedra, Spain) to preserve them.[23] The fucoxanthin powder extract was produced by water and ethanol extraction. These seaweed species were chosen on the basis of abundance in Western European waters and potential suitability as ingredients in the formulation of new products. The fucoxanthin extract was included for comparative purposes.

Sample preparation

Dried seaweeds were milled to a particle size of 1 mm by means of a mechanical grinder. Milled samples were stored vacuum packaged in a − 20 °C freezer for no longer than 30 days.

Compositional analysis

The moisture content of 2 g of seaweed was determined (Table 1) by drying the sample in a preheated (135 °C) oven for 2 h.[24] The fat content of the seaweed (5 g) was determined using the Foss Soxtec Avanti 2055 Manual system (Foss, Hillerød, Denmark). Seaweed samples were inserted into 33 mm × 80 mm extraction thimbles and extracted with 80 mL of boiling chloroform : methanol (2:1) for 30 min. Subsequently, thimbles were presented in the rinsing position for an additional 38 min. The extraction cups were removed and solvent was evaporated at 103 °C in an oven until a constant weight was obtained. The fat content was determined gravimetrically.

The protein content of 0.5 g of seaweeds was measured (Table 1) using the Kjeldahl method.[25] On completion, the content of the receiver flask was titrated with 0.1 M hydrochloric acid until the green color reverted back to the original red color. Finally, the protein was calculated using a nitrogen factor of 6.25. All analysis was performed in duplicate.

The ash content of seaweed was determined (Table 1) in duplicate by a muffle furnace (Nabertherm GmbH, Lilienthal, Germany),[26] which was pre-heated to 600 °C. Approximately 5 g of blended sample was weighed into porcelain dishes and placed into the muffle furnace. Samples were heated for 2 h until a white

Table 1. Composition of five dried seaweeds and a seaweed extract

	HE	UP	PU	PP	AE[a]	LJ	
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD	*P*-value
% Moisture	16.31 ± 0.43^{a}	10.77 ± 0.07^{b}	8.99 ± 0.22^{c}	10.04 ± 0.38^{b}	11.79 ± 0.12^{d}	6.68 ± 0.6^{e}	<0.001
% Fat	1.42 ± 0.17^{a}	2.30 ± 0.12^{b}	1.13 ± 0.03^{a}	1.69 ± 0.17^{a}	2.30 ± 0.07^{c}	0.38 ± 0.03^{d}	<0.001
% Protein	5.84 ± 0.44^{a}	9.02 ± 0.55^{b}	33.57 ± 1.4^{c}	19.96 ± 1.11^{d}	8.82 ± 0.31^{e}	0.6 ± 0.14^{f}	<0.001
% Ash	35.92 ± 0.45^{a}	57.36 ± 0.35^{b}	18.94 ± 0.31^{c}	28.49 ± 0.75^{d}	35.03 ± 0.49^{e}	5.93 ± 0.29^{f}	<0.001
% Salt	15.16 ± 0.25^{a}	36.77 ± 1.93^{b}	5.88 ± 0.13^{c}	16.34 ± 1.06^{a}	17.31 ± 0.21^{d}	-	<0.001

HE, *Himanthalia elongata*; UP, *Undaria pinnatifida*; PU, *Porphyra umbilicalis*; PP, *Palmaria palmata*; AE, *Alaria esculenta*; *Laminaria japonica* (fucoxanthin extract); M, mean; SD, standard deviation. $^{a\text{-}f}$, mean values in the same row bearing different superscripts indicate significant difference ($P < 0.05$) between the seaweed species.

[a] Halimah *O. Mohammed* HO *et al.* Characterization of the nutritional and bioactive properties of brown and red Irish seaweeds for potential use as functional ingredients in processed meat products. *47th Annual Food Science and Technology Conference* Cork, Ireland; 2018. (2018).

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ash was obtained, at which time they were put in a desiccator to cool down. The ash content was calculated by the weight of the dishes before and after sample introduction.

The salt content of seaweed was ascertained (Table 1), in duplicate by titration using silver nitrate (0.1M). Silver nitrate solution was standardized against 0.100% sodium chloride solution. Two grams of sample were inserted into the muffle furnace to create ash for ash content analysis. Ash was washed into conical flasks with 20 mL distilled water and 2 mL of added chromate indicator. The flasks were potentiometrically titrated with 0.1 N silver nitrate from a clear yellow to an opaque light orange (+255 mV) after cooling to room temperature. Blank titration was performed using 20 mL distilled water.

Extraction and volatile compounds analysis

Thermal desorption (TD)

The extraction of 10 g of dried milled seaweed by thermal desorption gas chromatography – mass spectrometry TD (GC–MS) was performed as previously described in Garicano Vilar *et al.* (2020)[27] for 40 min at 72 °C using a micro-chamber/thermal extractor (Markes International Ltd, Llantrisant, UK). The set of check standards used were 1-butanol, dimethyl disulfide, butyl acetate, cyclohexanone, benzaldehyde, and 2-phenyl-D5-ethanol, at 0.5 g kg^{-1}.

Headspace Solid-Phase microextraction (HS-SPME)

Headspace solid-phase microextraction analysis was performed as described by Lamichhane *et al.* (2018),[28] except that 3 g of dried milled seaweed (or powder extract) was extracted for 20 min at 40 °C, and the mass range scanned was 35–350 amu. Batch processing of samples was carried out using MetaMS, an open-source pipeline for GC–MS-based untargeted metabolomics.[29] A multiphase solid phase micro-extraction (SPME) 50/30 µm divinylbenzene/carboxen™/polydimethylsiloxane (CAR/DVB/PDMS) (Agilent Technologies Ltd, Cork, Ireland) was used because the properties of the different phases are utilized to target chemical classes based on their polarity and volatility and molecular weight. Previous studies have shown that this fiber choice was suitable for the extraction of volatile compounds in seaweeds.[5,7]

Gas Chromatography-Olfactometry (GC-O) analysis for aroma-active compounds

The extraction of volatile compounds was carried out by HS-SPME using a Gerstel MPS autosampler (Anatune Ltd, Cambridge, UK). The same SPME fiber (DVB/CAR/PDMS) was used as described earlier. The fiber was pre-conditioned before use at 270 °C for 60 min. Seaweed samples (dried seaweed and extract) (3 g) were weighed in 20 mL amber SPME La-Pha-Pack headspace vials with magnetic screw caps and silicone/polytetrafluoroethylene (1.3 mm 45° Shore A) septa (Apex Scientific Ltd, Maynooth, Ireland). The SPME fiber was exposed to the seaweed headspace at 40 °C for 60 min at a depth of 1 cm. Injections were carried out in splitless mode, with the injector temperature at 250 °C. All samples were analyzed in triplicate.

The GC-O analyses were conducted on an Agilent 7890A GC coupled with an Agilent 5975C mass selective detector (MSD) (Agilent Technologies Ltd, Cork, Ireland). The GC was also equipped with a flame ionization detector (FID) and a Gerstel olfactory detection port ODP3 with heated mixing chamber (Anatune Ltd, Cambridge, UK). A humidifying device was used to reduce nasal mucosa dehydration. Analyses were performed on a DB-624 column (20 m × 1.80 mm i.d. × 1 µm phase thickness). Helium was used as a carrier gas at a flow rate of 1.2 mL min^{-1} and nitrogen as auxiliary gas. The GC oven temperature was programmed to increase from 60 to 180 °C at a rate of 6 °C min^{-1}, with an initial hold time of 2 min, and from 180 to 220 °C at a rate of 15 °C min^{-1}, with a final hold time of 5 min. The total run time was 29.66 min. Column effluent was split equally between the FID, the olfactory port, and the MSD. The transfer line into the MSD was kept at 260 °C. The FID temperature was set at 300 °C, with an air flow of 400 mL min^{-1}, a H_2 fuel flow of 30 mL min^{-1}, and a N_2 makeup flow of 25 mL min^{-1}. The sniffing port and its exit were maintained at 150 °C and 40 °C, respectively.

A panel of three sensorial assessors (age 26–39 years) carried out the sniffing of the volatile compounds of seaweeds extracted by SPME. Sniffing time was approximately 30 min and each assessor carried out one session per day. The panelists were asked to rate (i) the intensity of the eluted aroma using a four-point category scale (1 = weak, hardly recognizable odor; 2 = clear but not intense odor; 3 = intense odor; 4 = very intense odor), recorded by a Gerstel OID Interface/ODP-Recorder (Anatune Ltd, Cambridge, UK), and (ii) the odor perceived, by voice recording. The odorants taken as significant were the ones that at least two assessors were able to detect. None of the assessors was anosmic, as tested by the Sniffin' Sticks test (Burghardt Messtechnik, Wedel, Germany) prior to the GC-O analysis.[30]

Tentative identifications were based on comparison of the mass spectra of unknown compounds against those of the National Institute of Standards and Technology (NIST), and by comparing the retention index value to values available in the literature and an in-house library. The odorants were also identified by comparison of their odors with Flavornet database (http://www.flavornet.org), The Good Scents Company database (http://www.thegoodscentscompany.com) and other specified publications. Retention indices for all detected volatile compounds were calculated using an n-alkane series (Merck, Arklow, Ireland) for both GC–MS and GC-O analysis.

Descriptive odor evaluation

Three panelists attended a focus group session on descriptive terms prior to the sensory analysis. The six samples were evaluated in one session, following the ISO 6658:2005 standard.[31] All samples were only assessed for odor attributes and analyzed in duplicate by each panelist. Mean scores for each attribute were calculated. The assessors quantified the attributes using a ten-point scale anchored to the left with 'not' and to the right with 'very'.[32] Two grams of each sample was put in 20 mL amber glass vials with screw caps (Apex Scientific Ltd) and the vials were kept in a HiSorb Agitator (Markes International Ltd, Llantrisant, UK) at 40 °C for 20 min at a rotational speed of 350 mn^{-1}, to ensure the accumulation of volatiles in the head space. Samples were provided randomly to panelist. The testing area used in this study complied with the ISO 8589:2007 standards.[33]

Data analysis

Normality and homogeneity were examined and a one-way analysis of variance (ANOVA) with the Bonferroni test for *post hoc* analyses was carried out to assess statistical differences between seaweed species in terms of composition and abundance values of volatile compounds, at a significance level of $P = 0.05$, using the SPSS 24.0 statistical package (IBM Corp., Armonk, NY, USA).

The volatile profile was analyzed using R software (RStudio, Inc., Boston, MA) for statistical computing and graphics. Principal

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component analysis was carried out to visualize the differences in volatile aroma composition detected by GC–MS, GC-O analysis, and sensory panel results, and to elicit the main factors contributing to the differences between the six samples.

In addition, volatile compounds and odor intensity values were further analyzed using ANOVA-partial least squares regression (APLSR) with Unscrambler X Software, version 10.3 (CAMO ASA, Trondheim, Norway) (see Fig. S1 in the supplementary material).

RESULTS AND DISCUSSION

Physiochemical analysis

The composition of the five seaweeds and the extract are summarized in Table 1. The moisture content was statistically different ($P < 0.01$) among all species, with the exception of *U. pinnatifida* and *P. palmata*. The data resemble the proximate composition of three brown seaweeds analyzed by Lorenzo *et al.*,[34] who reported that moisture content ranged from 7.95% in *B. bifurcata* to 11.2% in *A. nodosum* and *F. vesiculosus*. Rodrigues *et al.*[23] also reported similar moisture content: 9.6–10.9% in brown seaweeds and 8.0–11.8% in red seaweeds.

According to Ibañez *et al.*,[35] brown seaweed species (Phaeophyceae) are characterized by a lower protein content in comparison to red seaweed species (Rhodophyta). In effect, the red seaweeds (*P. umbilicalis* and *P. palmata*) presented a higher proportion of protein than the brown seaweeds (Table 1). The protein content was statistically different ($P < 0.001$) among all species tested. These findings are similar those of Lorenzo *et al.*,[34] where the protein content of brown species ranged from 8.7 to 13.0%. However, Rodrigues *et al.*[23] reported higher (14.4 to 16.9%) protein content for brown species and lower protein content (20.2 to 23.8%) for red species.

Seaweeds are well known for their low fat content, but values vary considerably between studies. A range of 1.1 and 2.3% of total fat was found in our samples (Table 1). The fat content of red seaweed did not have any significant differences, but significant differences were evident for green seaweed ($P < 0.01$). Higher fat content of 0.9 and 0.6% were reported in red species of *U. pinnatifida* and *Gracilacia gracilis*, respectively, by Rodrigues *et al*[23] Higher fat content was also observed in brown species in this study compared to those reported by Jard *et al.*[36] for *Sargassum muticum*.

Higher levels of ash are associated with higher amounts of minerals.[37] The ash content of *P. umbilicalis* and *P. palmata* was lower than that of brown seaweed samples. *Undaria pinnatifida* had the highest ash content (Table 1). Greater variability in total ash content was observed in brown seaweed than in red seaweed. The differences between the seaweed species were all statistically significant ($P < 0.001$), even between red and brown seaweeds. The ash content values observed in this study are in agreement with published values for *Grateloupia turuturu* (18.5%) by Denis *et al.*[38] and for *G. gracilis* (24.8%) by Rodrigues *et al.*[23] In contrast, higher values of ash content were observed in brown species in this study than other studies.[23,34] The salt content of all seaweeds followed the same trend as the ash content (Table 1), although no statistically significant difference was observed between *H. elongata* and *P. palmata*.

Volatile compounds identification

A total of 117 (Table S2) and 109 (Table S3) volatile compounds were detected in the five seaweed species and in the seaweed extract by TD and HS-SPME, respectively. These numbers were lower than the 151 volatile compounds identified by López-Pérez *et al.*[7] in seven species of dehydrated edible seaweeds. The number of volatile compounds identified in each individual seaweed species by TD and HS-SPME, respectively, were 75 and 61 compounds for *H. elongata*, 76 and 58 for *U. pinnatifida*, 65 and 58 for *P. umbilicalis*, 78 and 76 for *P. palmata*, 72 and 60 for *A. esculenta*, and 67 and 53 for the fucoxanthin extract. These numbers were lower than the 129, 140, 131, and 136 volatile compounds found by López-Pérez *et al.*[7] in *H. elongata*, *U. pinnatifida*, *P. umbilicalis*, and *P. palmata*, respectively, but higher than the 23 volatiles in *P. palmata* samples[9] or the 26 and 24 volatile compounds in *Laminaria* spp and *U. pinnatifida*, respectively.[13] The differences may be due to a myriad of reasons; species, geographical area of growth, harvesting, storage, and volatile extraction conditions amongst others. As previously mentioned, no single extraction technique can provide a complete volatile profile as they have an inherent bias due to the different adsorbent and absorbent properties of the different phases, molecular sieve characteristics, mechanisms of extraction and the physical parameters used, etc. In this study we carried out HS-SPME at 40 °C and extracted for 20 min, and we carried out TD at 72 °C for 40 min in an attempt to extract more compounds as much more sorbent capacity exists for TD than HS-SPME and larger amounts of sample were used (10 g versus 3 g) (Fig. S4 and S5). As mentioned, the properties of the sorbent materials used in these techniques are different and will therefore extract and concentrate different VOCs. The TD tubes contained Tenax Carbograph, which is useful for the detection of VOC between C_3 and C_{30} of different volatilities (typically 50–450 °C) and polarity. Limitations occur for some low molecular-weight compounds lower than C_3, especially if they are polar, such as carboxylic acids, some aldehydes, and alcohols.[39] The SPME fiber (DVB/CAR/PDMS) is useful for a wide range of volatiles and semi-volatiles between C_3-C_{20}. This fiber affords a greater extraction efficiency for many VOC, in comparison to other fibers (PDMS-DVB, CAR-PDMS, PDMS or polyacrylate) and is particularly useful for very volatile VOCs but less useful for heavier VOCs such as lactones.[40–42] It is therefore useful to utilize different VOC extraction / concentration techniques to achieve a more representative VOC profile.

From the TD analysis the highest numbers of volatile compounds were, in decreasing order; 23 alcohols, 20 ketones, 20 aldehydes, nine acids, eight esters, seven furans, six benzenes, five pyrazines, five terpenes, four sulfur compounds, three ethers, three lactones, two terpenes, two pyridines, one phenol and one chlorine, with HS-SPME detecting 21 ketones, 20 aldehydes, 16 alcohols, nine acids, nine benzenes, seven esters, six furans, six pyrazines, five terpenes, three sulfur compounds, three lactones, two ethers, one phenol and one chlorine. The different volatiles detected by both extraction techniques highlight the merits of using more than one extraction technique to encompass a larger volatile profile.

Overall, terpenes were most prominent in *H. elongata*; acids and lactones in *U. pinnatifida*; sulfur compounds in *P. umbilicalis* (HS-SPME extraction only); aldehydes, esters, ketones, pyrazines, and pyridines in *P. palmata*; alcohols, ethers, and furans in *A. esculenta*, and benzenes and phenol were predominant in fucoxanthin extract.

Descriptive odor analysis

Descriptive odor analysis was conducted on the five different species of seaweed and the seaweed extract (Fig. 1). The samples were classified and characterized by 'rancid, oily / fatty, pungent',

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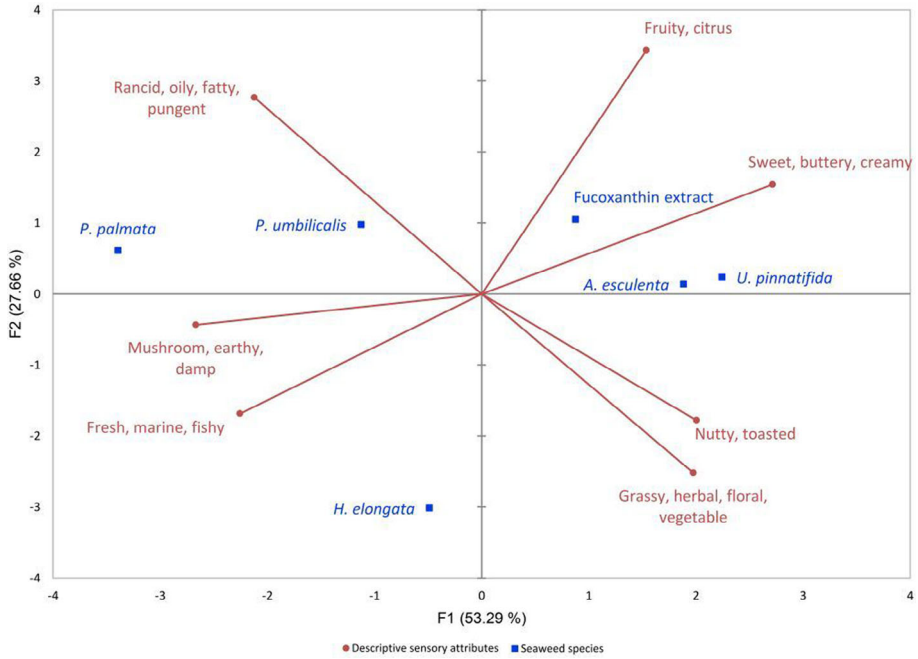


Figure 1. Spider diagram of the sensory evaluation of five seaweeds (*H. elongata*, *U. pinnatifida*, *P. umbilicalis*, *P. palmata*, and *A. esculenta*) and one seaweed extract (fucoxanthin extract from *L. japonica*). Average scores are shown according to quantitative descriptive odor attributes evaluated by three panelists.

'fruity, citrus', 'grassy, herbal, floral, vegetable', 'fresh, marine, fishy', 'nutty, toasted', 'mushroom, earthy, damp' and 'sweet, buttery, creamy' aroma. A PCA was produced to visualize the differences in the aroma attributes between the species and the extract (Fig. 2). The first two principal components of the PCA were able to explain 81% of the variance (Fig. 2). Globally, *H. elongata* and *P. palmata* were mostly characterized by a 'fresh, marine, fishy' aroma; *U. pinnatifida* and *A. esculenta* by 'grassy, herbal, floral, vegetables' notes; *P. umbilicalis* by 'mushroom, earthy, damp' and 'rancid, oily, fatty' notes and fucoxanthin extract by 'nutty, toasted' aroma (Fig. 2).

Odor characteristics of aroma active compounds determined by HS-SPME-GC-O

In total 41 aroma active volatiles were identified and five unidentified aroma-active compounds were perceived by panelists, consisting of acids, alcohols, aldehydes, ethers, furans, ketones, and pyrazines (Table 2). If a volatile compound(s) is present at a concentration above its odor threshold but below its limit of detection it cannot be identified but its aroma will be perceived by GC-O. Quite a few of the compounds were also summarized as having odor descriptors different or partially different from the referenced descriptors. However, this is also not unusual as odor descriptors can be influenced by the concentration (odor descriptors can change based on concentration for some volatile compounds), the matrix effect (the composition of the sample can influence the release of volatiles into the headspace), and differences in the abilities of the human assessors due to genetic and cultural differences.[43]

Palmaria palmata had the strongest odor of all samples, as the sum of the olfactory scores was the highest at 54.1[44] (Table 2). On the other hand, the fucoxanthin extract had the lowest score (8.5), resulting in a rather bland aroma profile that was dominated by 'bakery, buttery, sweet, fatty' aromas. This is possibly due to the additional processing required to produce the extract. In addition to heptanal, and taking into account the olfactory scores for each compound individually, (*E,Z*)-2,6-nonadienal (fresh, cucumber, grass, green) and sulcatone (woody, green, grass, celery, vegetable) appear to be important aroma compounds for *H. elongata* and *U. pinnatifida*. *Porphyra umbilicalis* was characterized by three unidentified compounds with (i) 'toasted, yeast, fermented, roasted, nutty', (ii) 'pyrazine, grass, boiled potato', and (iii) 'floral, geranium, green, grass, damp, mouldy' aromas. *P. palmata* was dominated by 2,6-dimethyl-pyrazine (baked, toasted, roasty, caramel), and an unidentified component with 'pyrazine, grass, boiled potato' aroma and 3-heptanone (fruity, butter, oily). *Alaria esculenta* was characterized by 1-octen-3-ol (mushroom, earthy), 3-heptanone (fruity, rancid, butter, oily), and benzaldehyde (green, grass, vegetable, herbal, almond). 2-Furanmethanol (cheese, butter, buttermilk, rancid) and 2-methyl-nonane (backed, toasted) appeared to be characteristic aroma notes for only the fucoxanthin extract but at low (<3) intensities.

As mentioned, GC-O analysis showed that 1-octen-3-ol (mushroom, mineral, hay) and heptanal (herbal, grassy) were common in all samples but differed in their intensities. The compound 1-octen-3-ol contributes to 'mushroom' and 'mushroom-metallic' notes.[20,45] Moreover, 1-octen-3-ol has been shown to contribute to the formation of seafood aroma.[45] Heptanal contributes to 'green' and 'fresh' aroma.[46] It has also been described as having 'fat', 'rancid', 'citrus' aromas.[47] Heptanal, which was the potent aroma compound for all samples in this study, exhibited a 'fatty, oily, fishy, seaweed, lake water' aroma and appears to contribute to the characterizing notes of the fish, seaweed, oil aromas of seaweed. Many short chain aldehydes (e.g., 2-octenal) can also provide several aromas to food matrices (fatty, green, woody, fatty, nutty, floral, citrus, waxy, and sweet) depending on the number of carbon atoms and the degree of saturation.[45]

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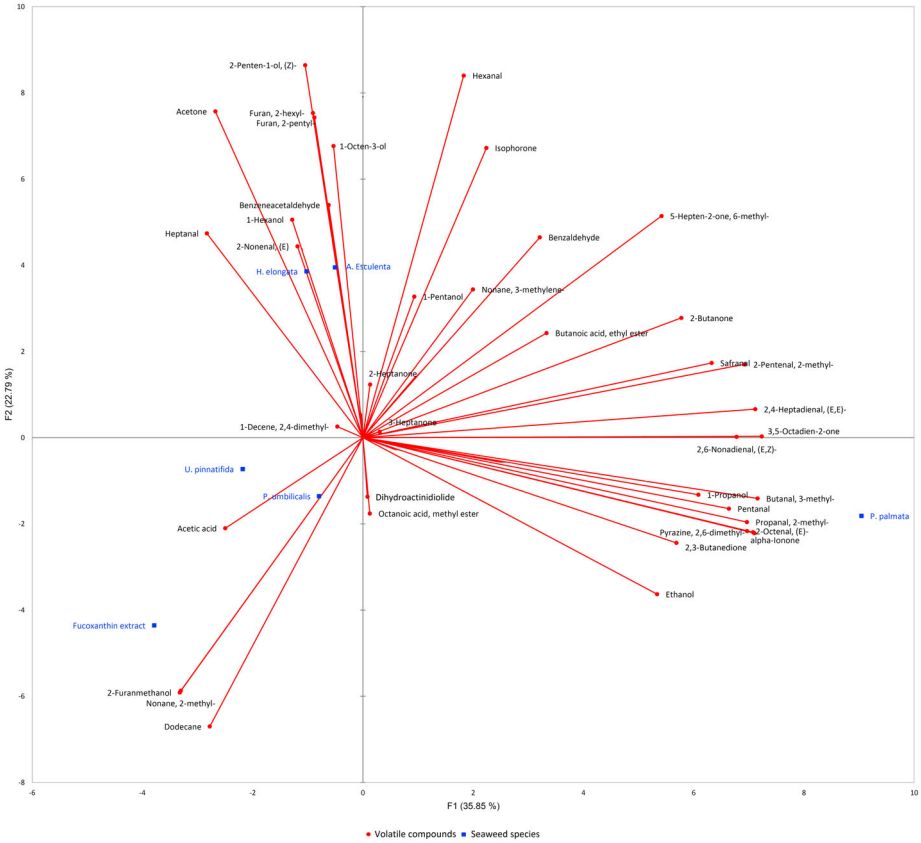


Figure 2. Principal component analysis scores plot of aroma evaluation of five seaweed samples (*H. elongata*, *U. pinnatifida*, *P. umbilicalis*, *P. palmata*, and *A. esculenta*) and one seaweed extract (fucoxanthin extract from *L. japonica*), for the first two principal components (PC1 and PC2). Average scores are according to quantitative descriptive odor attributes evaluated by three panelists.

Many volatile compounds in a food present low aroma intensity or no aroma and thus do not necessarily contribute to the overall odor.[48] Thus, high threshold compounds can present low odorant power, even if present at a high abundance. Conversely, low concentrations of low threshold compounds in the sample can present a high odor intensity.[19] It is therefore necessary to know if the extracted volatile compounds are actually contributing to the characteristic aroma.

A second PCA was performed on the seaweed samples in order to determine the relationships between the volatile aroma active compounds as determined by GC-O analysis in the different seaweed species (Fig. 3). The first two principal components explain 59% of the variance.

A clear discrimination exists between these samples based on their olfactometry assessment (Fig. 3). Both *H. elongata* and *A. esculenta* are quite similar, on the positive side of F2 and the negative side of F1. These samples are associated with heptanal (fatty, oily, fishy, seaweed, lake water), (*E*)-2-nonenal (fresh, lemon, grassy, nuts), 1-hexanol (lemon, citrus, grass), benzeneacetaldehyde (tea, floral), 1-octen-3-ol (mushroom, mineral, hay), 2-hexyl furan (fruity, floral, green, tea), 2-pentyl furan (butter, toasted, herbal), acetone (sulfur, boiled corn, rocket), (*Z*)-2-penten-1-ol (sweet, cherry, fruity, candy), 1-pentanol (cotton candy, fruity) and 2-heptanone (grassy, plastic). Only some of the individual odor descriptors match the overall aroma characteristic by hedonic assessment; however, during aroma evaluation the panelists are sniffing a combination of all these odors rather than individual odors by GC-O, thus combined odors may be perceived very differently.[49] It is worth noting that odor thresholds and abundances are the main factors that impact on odor perception. It seems plausible that heptanal must be a key odorant in these species as it has an odor similar to their aroma attributes. Heptanal also has a mid- to low odor threshold, but a high odor intensity score (Table 2). The intensity scores for (*E*,*Z*)-2,6-nonadienal (*Z*)-2-penten-1-ol in *P. umbilicalis*, *U. pinnatifida* and the fucoxanthin extract were all on the negative side of F1 and F2 (Fig. 3). These were all associated with 'fruity, citrus', 'sweet, buttery, creamy' aroma attributes. Both *P. umbilicalis* and *U. pinnatifida* were strongly associated with acetic acid (vinegar) and *P. umilicalis* was also strongly associated with methyl octanoate (fermented, fresh, green). It appears that methyl octanoate contributes to the overall aroma character of *P. umilicalis* as its aroma is similar. The fucoxanthin extract was differentiated from all the other samples. It was associated more with 2-furanmethanol (cheese, butter, rancid), 2-methyl-nonane (toasted), and dodecane (soap, glue) and less with acetic acid or methyl octanoate. The individual aromas of these volatiles, however, do not match the overall aroma characteristics. *Palmaria palmata* was also discriminated from all the other samples on Fig. 3, on the positive side of F1 and the negative side of F2. It was associated most with 'mushroom, earthy, damp' and 'rancid, oily, fatty, pungent' aroma attributes but also with a very wide range of volatiles: 3-methylbutanal (vanilla, sweet, floral, butter), pentanal (caramel, sugar, spicy, butter), 2-methyl propanal (vanilla, floral), 2,6-dimethyl

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Table 2. Aroma-active compounds detected in five dried species of edible seaweeds and a seaweed extract by GC-O

Compound	RI[a]	LRI[b]	IM[c]	Odor[d]	Odor intensity[e] HE	UP	PU	PP	AE	LJ	Odor ref. [a-i]	Odor threshold (ppb) [j-n]
ACIDS												
Acetic acid	687	690	A, C	Vinegar	1.6	2.6	-	-	-	-	Vinegar, sharp, sour [a]	480–1000 [j]
Propanoic acid	780	784	A, C	Cheese, rancid, medical	-	2	-	-	-	-	Pungent, acidic, cheesy, vinegar, dairy [a]	20 000 [k]
ALCOHOLS												
Ethanol	483	506	A, C	Alcohol	-	1.3	-	-	0.6	-	Strong, alcoholic, ethereal, medical [a]	100 000 [k]
1-Propanol	610	615	A, C	Sweet	-	-	1.3	-	-	-	Alcoholic, fermented, tequila, musty, yeasty, sweet, fruity, apple, pear [a]	9000 [k]
2-Furanmethanol	931	939	B, C	Cheese, butter, buttermilk, rancid	-	-	-	-	-	2.6	Alcoholic, chemical, musty, sweet, caramel, bread, coffee [a]	8000 [L]
2-Penten-1-ol, (Z)-	825		B, C	Sweet, cherry, fruity, candy	3	-	-	-	-	-	Cherry, narcissus, fruity, green, phenolic, nasturtium, ethereal, medicinal, aldehydic, metallic [a]	Nf
1-Pentanol	817	815	A, C	Cotton candy, fruity	-	-	1.6	-	-	-	Pungent, fermented, bready, yeasty, fusel, winey, solvent [a]	4000 [k]
1-Hexanol	919	916	B, C	Lemon, citrus, grass	-	-	-	-	1	-	Fruity, alcoholic, sweet, green, pungent, ethereal, fusel, oily [a]	2500 [k]
1-Octen-3-ol	1028	1030	A, C	Mushroom, mineral, hay	2	2.3	2	2	3.3	-	Mushroom, earthy, green, oily, fungal [a-c]	1 [k]
ALDEHYDES												
Propanal, 2-methyl-	598	592	A, C	Vanilla, floral	-	-	1	1.3	-	-	Fresh, aldehydic, floral, pungent [a]	0.1–2.3 [k]
Pentanal	733	735	A, C	Caramel, sugar, spicy, butter	-	-	1.6	-	-	-	Fermented, bready, fruity, nutty, berry [a]	12–42 [k]
Butanal, 3-methyl-	694	692	A, C	Vanilla, sweet, floral, butter	-	-	1.3	2.3	-	-	Ethereal, aldehydic, chocolate, peach, fatty [a]	12 [m]
Hexanal	837	839	A, C	Herbal, grassy	2.3	-	1.6	1.6	-	-	Fresh, green, grassy, leafy, fruity, sweaty, woody, clean [a,c]	5 [k]
2-Pentenal, 2-methyl-	877		B, C	Sweaty, milk, rancid	-	-	-	2	-	-	Pungent, fruity, juicy, ripe, aldehydic, green, grassy, gassy [a]	290 [n]
Benzaldehyde	1034	1030	B, C	Green, grass, vegetable, herbal, almond	-	-	-	1.3	3	-	Almond, burnt sugar-like, cherry, bitter, sharp, fruity, sweet, bitter almonds [a,c-e]	350–3500 [k]
2,4-Heptadienal, (E,E)-	1074		B, C	Grassy, vegetable, cucumber	-	-	-	2.3	-	-	Fatty, green, oily, aldehydic, vegetable, cinnamon [a]	3600 [m]
Heptanal	942	943	A, C	Fatty, oily, fishy, seaweed, lake water	3.3	3.6	4	3.6	3.3	2	Fresh, aldehydic, fatty, green, herbal, wine-lee, ozone, citrus, rancid [a,c,f]	3–60 [n]
Benzeneacetaldehyde	1109	1119	A, C	Tea, floral	-	0.6	-	-	-	-	Harsh, green, honey, cocoa, sweet, floral, hyacinth, clover, rose, powdery, fermented [a,f,g]	4 [k]
2-Octenal, (E)-	1116		B, C	Green, grass, pepper, coffee	-	-	-	1.6	-	-	Fresh, cucumber, fatty, green, herbal, banana, waxy, leafy, nut [a,f]	3 [k]
2,6-Nonadienal, (E,Z)-	1216		B, C	Fresh, cucumber, grass, green	3.3	3	-	3	-	-	Green, cucumber, melon, fatty, vegetable, dry, violet, leaf [a,c]	0.01 [k]
2-Nonenal, (E)-	1218		B, C	Fresh, lemon, grassy, nuts	2.3	-	-	-	-	-	Green, cucumber, aldehydic, fatty, citrus [a]	0.08–0.1 [k]

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Table 2. Continued

Compound	RI[a]	LRI[b]	IM[c]	Odor[d]	Odor intensity[e] HE	UP	PU	PP	AE	LJ	Odor ref. [a-i]	Odor threshold (ppb) [j-n]
ETHERS												
Butanoic acid, ethyl ester	747	828	B, C	Fruity, cherry, strawberry, candy	-	2.6	2	2.3	-	-	Sweet, fruity, tutti frutti, juicy [a]	1 [k]
Octanoic acid, methyl ester	1151	1156	B, C	Fermented, fresh, green	-	-	1	-	-	-	Waxy, green, sweet, orange, aldehydic, vegetable, herbal [a]	200 [k]
FURANS												
Furan, 2-pentyl-	1009	1012	A, C	Fried, melted butter, toasted, herbal	2	-	-	1.3	-	-	Fruity, green, earthy, beany, vegetable [a,c]	6 [k]
Furan, 2-hexyl-	1386		B, C	Fruity, floral, green, tea	1.6	-	-	-	-	-	Floral, pulpy [h]	Nf
KETONES												
Acetone	565	533	A, C	Boiled corn, rocket	-	-	1	-	-	-	Solvent, etheral, apple, pear [a]	500 000 [k]
2,3-Butanedione	627	631	A, C	Butterscotch, sweet, yeast, butter, vanilla, custard	-	2.6	1	-	1.3	1	Buttery, sweet, creamy, caramellic, pungent [a,c,f]	2.3–6.5 [k]
2-Butanone	636	639	A, C	Butterscotch, sweet, vanilla, cream, fruity	1.3	-	-	2.3	-	-	Camporheous, fruity, ethereal, acetone, nauseating odor [a,c,e]	50 000 [k]
2-Heptanone	934	928	A, C	Plastic, grassy	-	-	-	1.3	1.6	-	Fruity, spicy, sweet, herbal, coconut, woody, sulfur, pungent, green [a,f]	14–3000 [k]
3-Heptanone	927		A, C	Fruity, rancid, butter, oily	2	2.6	-	3.3	3.3	-	Green, fatty, fruity [a]	Nf
3,5-Octadien-2-one	1131		B, C	Metallic, mineral	1.6	0.6	-	2	-	-	Fruity, fatty, mushroom [a]	0.15 [n]
Sulcatone	1032		A, C	Woody, green, grass, celery, vegetable	3.3	3	-	3	-	-	Citrus, green, musty, lemongrass, apple, cheesy, banana [a]	50 [k]
Isophorone	1108		B, C	Grapefruit, spicy, wet, rosy, floral, caramel, sugar	-	-	-	2	1.3	-	Cooling, woody, sweet, green, camphor, fruity, musty, cedar, wood, tobacco, leather [a]	200 [L] (air)
PYRAZINES												
Pyrazine, 2,6-dimethyl-	947	944	A, C	Backed, toasted, roasty, caramel	-	-	-	3.6	-	-	Cocoa, roasted, nutty, meaty, coffee [a]	400–1500 [n]
TERPENES												
Dihydroactinidiolide	1701		B, C	Damp, floral	-	-	-	1.6	-	-	Ripe, apricot, fruity, berry, woody [a]	Nf
α-Ionone	1500		B, C	Grassy, lemon, citrus, sugary, fruity	-	-	-	1.6	-	-	Sweet, woody, floral, violet, orris, tropical, fruity [a]	0.6–10 [n]
OTHERS												
Safranal	1260		B, C	Grassy, vegetable, anise, fennel	1.3	-	-	1.6	1	-	Fresh, herbal, phenolic, metallic, rosemary, tobacco, spicy, woody, camphoreous, medicinal, powdery, herbal [a]	Nf
Nonane, 3-methylene-	991		B, C	Incense, spices, earthy, floral, sweet, cherry, strawberry	-	-	1.3	2.3	-	-	Nf	Nf
Nonane, 2-methyl-	965		B, C	Baked, toasted	-	-	-	-	-	1.3	Nf	Nf
1-decene, 2,4-dimethyl-	1229		B, C	Grass, green	-	-	2.3	-	-	-	Nf	Nf
Dodecane	1199		B, C	Soap, glue	2	1.3	-	1.3	-	-	Gasoline-like [i]	Nf
Unidentified-1	961		C	Toasted, yeast, fermented, roasted, nutty	-	-	3	-	-	-		
Unidentified-2	969		C	Pyrazine, grass, boiled potato	-	-	3	3.6	-	-		
Unidentified-3	1032		C	Floral, geranium, green, grass, damp, mouldy	-	-	3	-	-	-		
Unidentified-4	1143		C	Burning wood, hay, grass	-	-	-	-	1.6	-		
Unidentified-5	1226		C	Rubber, plastic	-	-	-	-	-	1.6		

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Table 2. Continued

Compound	RI[a]	LRI[b]	IM[c]	Odor[d]	Odor intensity[e] HE	UP	PU	PP	AE	LJ	Odor ref. [a-i]	Odor threshold (ppb) [j-n]
Total odor intensity					32.9	28.1	32	54.1	21.3	8.5		

HE, *Himanthalia elongata*; UP, *Undaria pinnatifida*; PU, *Porphyra umbilicalis*; PP, *Palmaria palmata*; AE, *Alaria esculenta*; LJ, *Laminaria japonica* (fucoxanthin extract).
[a] Retention index (RI) calculated from GC-O results on a DB-624 UI column.
[b] Retention index found in the literature (LRI) for a DB-624 UI column.
[c] Identification method (IM): A, identification based on NIST mass spectral database, RI values from the literature and an in-house library created using authentic compounds with target and qualifier ions and linear RI for each compound; B, when only B or RI values were available, it must be considered as a tentative identification; C, identification with GC-O.
[d] Odor description given by three assessors during GC-O analysis. -, not detected.
[e] Mean odor intensity for each seaweed species evaluated by sniffers. From 1 = low to 4 = high.
[f] Odor descriptors found in the literature: **a** [http://www.thegoodscentscompany.com/index.html], **b** [Isleten Hosoglu M. *Food Chem* 240:1210–1218 (2018)], **c** [Narain N. *Examines Mar Biol Oceanogr* 2:195–201 (2018)], **d** [Minteguiaga M *et al. J Sep Sci* 38:3038–3046 (2015)], **e** [Flament I. 1st ed. John Wiley & Sons Ltd., ed. Chichester, England; 2002], **f** [Dong L *et al. J Sci Food Agric* 95:915–921 (2015)], **g** [Lasekan O *et al. CYTA - J Food* 14:154–161 (2015)], **h** [de Sousa Galvão M *et al. Food Res Int* 44:1919–1926 (2011)], **i** [Verma DK *et al.* 1st ed. Verma DK, Srivastav PP, eds. Sci. Technol. Aroma, Flavor, Fragr. Rice. Oakville, Canada: Academic Press, Inc; 2019]. Nf, not found.
[g] Odor threshold values in water (unless otherwise designated) found in the literature: **j** [New Jersey Department of Health. Hazardous Substance Fact Sheet Acetic Acid. 2016], **k** [http://www.leffingwell.com/odorthre.htm], **l** [U.S. National Library of Medicine and National Center for Biotechnology Information, 2016], **m** [American Society for Testing and Materials. 2nd ed. Fazzalari FA, ed. Baltimore, Md.: American Society for Testing and Materials; 1978], **n** [Burdock GA. *Fenaroli's Handbook of Flavor Ingredients, Fourth Edition*, 2001]. Nf, not found.

pyrazine (backed, toasted, roasty, caramel), α-ionone (grassy, lemon, citrus, sugary, fruity), (*E*)-2-octanal (green, grass, pepper, coffee), 2,3-butanedione (butterscotch, sweet, yeast, butter, vanilla, custard), ethanol (alcoholic), 1-propanol (sweet), methyl octanoate (fermented, fresh, green), (*E,Z*)-2,6-nonadienal (fresh, cucumber, grass, green), 3,5-octadien-2-one (metallic, mineral), and dihydroactinidiolide (damp, floral). It appears that 3,5-octadien-2-one is likely a major contributor to the aroma of *P. palmata* due to its odor characteristics and intensity score (Table 2).

Panelists in the study by Peinado *et al.*[50] used 'honey-like' odor, 'herbal' odor, 'seaweed-like' odor attributes to describe five different species of brown edible seaweeds (*Laminaria digitata*, *Ascophyllum nodosum*, *Pelvetia canaliculata*, *Fucus vesiculosus*, and *Fucus spiralis*). A 'seaweed-like' aroma was, in general, the attribute with the highest score – which could be expected as it is the attribute most related to 'seafood like'. Only the Laminaria species extract was significantly different from all the others in terms of aroma, being the one with the strongest 'seaweed-like' aroma, and the mildest 'honey-like' aroma. Yamamoto *et al.*[1] asked panelists to evaluate aroma attributes like animalic, floral, spicy, fatty, green note, marine-like, fresh (watery), powdery and leather-like, in *Ulva prolifera*, *Ulva linza* and *Monostroma nitidum*. They reported that, depending on the Japanese prefecture where the seaweed originated, seaweeds were evaluated differently in respect of its green-note, marine-like, fresh and powdery aroma. Some further studies analyzed the aroma compounds produced by marine microalgae species[20,45] and flagellates,[21] and concluded that they could be described by different aroma characteristics, but microalgae findings are not relevant to this research.

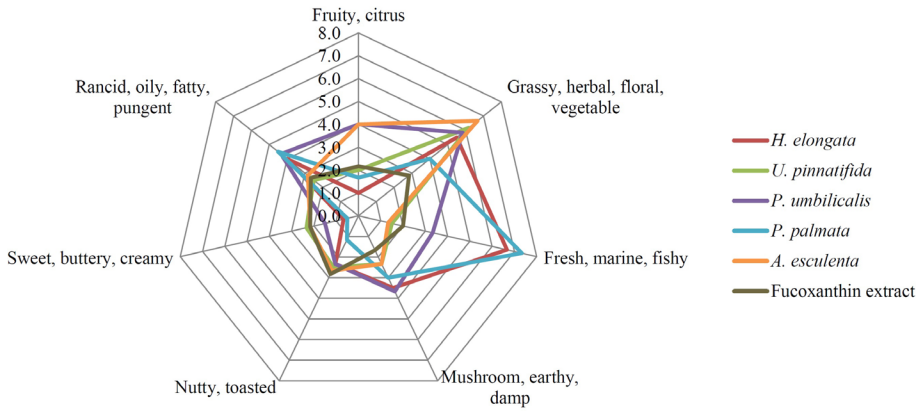


Figure 3. Principal component analysis scores plot depicting the distribution of aroma compounds detected by gas chromatography-olfactometry (GC-O) analysis in five seaweed species (*H. elongata*, *U. pinnatifida*, *P. umbilicalis*, *P. palmata*, and *A. esculenta*) and one seaweed extract (fucoxanthin extract from *L. japonica*), for the first two principal components (PC1 and PC2).

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The volatile profiles of *H. elongata*, *U. pinnatifida*, *A. esculenta*, *L. japonica* fucoxanthin extract, *P. umbilicalis*, and *P. palmata* were described, and some for the first time in this study. This research, based on SPME-GC–MS and TD-GC–MS, has revealed that volatile compounds differ intensely in the five varieties of dried seaweed and the seaweed extract. We also reported key odorants of the different commercial brown and red seaweed samples. This work shows the potential of GC–MS and GC-O coupled with multivariate analysis (PCA) to discriminate between samples with different origins, based on their volatile profiles, and to form the basis for future development of authentication methods and characterization of seaweed-containing products.

Little information is available on species such as *H. elongata*, *U. pinnatifida*, *P. umbilicalis*, and *P. palmata*, which are common brown and red species in abundance around the coastlines of Western Europe. For some species such as *A. esculenta* and *L. japonica* no details on their volatile profiles or odor characteristics have been published before. This study therefore addresses significant recent developments in the current understanding of the volatile profile of these seaweeds and the key aroma compounds that contribute to their unique odors. This characterization of volatiles in seaweeds may help to develop new products that are more acceptable to consumers who are less familiar with seaweed, especially as seaweeds are of growing importance in Western diets, and most recently as components of functional foods. The work has contributed to an understanding of volatile profiles in seaweeds and in particular our chemosensory knowledge of seaweed odor. Further work is required in relation to VOC changes that occur in the processing and storage of seaweed species as extracts and in their application in food in relation to sensory perception.

LIMITATIONS

Gas-chromatography – olfactometry alone does not allow a final conclusion on the contribution of a single compound to the overall aroma. A reason for this is that the entire amount of a compound present in a sample is volatilized during GC-O, whereas, from a food, only the amount present in the headspace above the food is available for the nose receptors and thus is odor-active. Odor activity values (OAVs) are helpful to understand the contribution of aroma compounds because they correlate quantitative data with odor thresholds in a matrix and thus address the influence of the matrix on the volatility of a given odorant.[47]

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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Effect of salt reduction and inclusion of 1% edible seaweeds on the chemical, sensory and volatile component profile of reformulated frankfurters

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ABSTRACT

The optimization of processed meats through salt replacement using edible seaweeds may reduce the risk of chronic disease through reduction in dietary sodium. We investigated the impact of the inclusion of four selected seaweeds (1% *w*/w) in reformulated frankfurters in which salt addition and pork fat content was reduced by 50% and 21%, respectively, and where pork loin (*longissimus dorsi* muscle) was increased by 6%, compared to a Control. Two different types of red (*Porphyra umbilicalis* and *Palmaria palmata*) and brown (*Himanthalia elongata* and *Undaria pinnatifida*) edible seaweeds were evaluated. The reformulated frankfurters containing added seaweed were lower in ash, higher in moisture, protein and darker in colour and had altered textural properties in comparison to the Control; mainly less hard and less chewy. The volatile and sensory profiles of the reformulated frankfurters differed from the Control. However, the reformulated frankfurters with the inclusion of *H.elongata* were the most promising, although further work is required to optimise the formulation.

1. Introduction

Irish consumers are concerned about dietary intake of fat, salt and cholesterol in pork products and are demanding healthier alternatives (Donoghue, Duffy, Mullane, Smyton, & Talbot, 2008). There is a particular interest in the consumption of breakfast and frankfurter sausages in Ireland, but these products typically contain high fat and sodium levels with lipid profiles that diverge from dietary objectives for optimal health (Roohinejad et al., 2017). However, these products are of considerable economic importance and are widely accepted in certain sectors of the population (López-López, Cofrades, Yakan, Solas, & Jiménez-Colmenero, 2010). According to the National Adult Nutrition Survey (NANS) (McCarthy, McCarthy, & McGrath, 2017), 13% of the Irish population are considered as 'processed pork indulgers', consuming five times more sausages, bacon and pudding than other meat consumer segments. Pork eaters derive 28% of their energy intake from meat in their diet and have fat and salt intakes above that recommended for a healthy diet. Thus, from a public health perspective, there is a need to decrease salt (Trieu et al., 2017) and fat (Briggs, Petersen, & Kris-Etherton, 2017) intake but this is difficult without comprising sensory quality.

Processed meat refers to meat (mainly pork or beef, but may also contains other meats or meat by-products) that have been transformed through salting, curing, fermentation, smoking or other processes to enhance flavour or improve preservation, and often contain high quantities of minced fatty tissues (e.g. sausages) (Domingo & Nadal, 2017). Processed meat products with enhanced composition have been formulated both by reducing the concentrations of salt, fat, cholesterol and nitrite (unhealthy compounds) and by promoting the presence of healthy compounds (e.g. bioactive compounds) (Arihara, 2006; Fernández-Ginés, Fernández-López, Sayas-Barberá, & Pérez-Alvarez, 2005). Meat products adapted by the food industry using novel processing methods based on reformulation, reducing salt and fat and adding healthier components, are key strategies (e.g. modification of the animal's diet to produce raw material with healthy ingredients) under investigation (Beriain, Gómez, Ibáñez, Sarriés, & Ordóñez, 2018). Changes in formulation may have an impact on quality and palatability and must be accepted from the consumer perspective in order to attain general acceptance and market share. Frankfurters, burgers and patties, represent processed meat products where compositional modification is more easily attained to improve their nutritional value and health properties (Jiménez-Colmenero et al., 2010).

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Seaweeds are good sources of nutrients and bioactive phytochemicals and have gained importance in Western diets, and most recently as components of functional foods (Gupta & Abu-Ghannam, 2011; Qin, 2018). All edible seaweeds offer considerable potential as natural sources of important components with many different biological activities (e.g. antioxidant (Zhao et al., 2018) and antihypertensive activities (Cofrades et al., 2010)), making them suitable candidates as food ingredients for promoting health. Although reducing salt and fat in processed meats by the addition of seaweed may provide additional functional properties, such as nutritional (less calories and saturated fatty acids), physicochemical and textural properties (Roohinejad et al., 2017), seaweed addition may also result in decreased consumer acceptability, due to the unique flavour of seaweed. The chemical composition of seaweeds is species dependent (Cofrades et al., 2010). The two red edible seaweeds, Nori (*Porphyra umbilicalis*) and Dulse (*Palmaria palmata*) contain higher quality proteins and proportionally larger amounts of all amino acids than the edible brown species, Sea Spaghetti (*Himanthalia elongata*) and Wakame (*Undaria pinnatifida*) (Cofrades et al., 2010). The lipid content of *H. elongata* is higher than that of *P. umbilicalis*, but similar to *U. pinnatifida* (Cofrades et al., 2010). The two brown species exhibit higher saturated fatty acid contents, than red species, but the main difference as regards to fatty acid profiles is the makeup of monounsaturated and polyunsaturated fatty acids (Cofrades et al., 2010). *H. elongata* contains the highest proportion of soluble and total dietary fiber, whereas *U. pinnatifida* is richer in insoluble fiber (Cofrades et al., 2010). All seaweeds are rich in ash content compared to land plants. *H. elongata*, *U. pinnatifida* and *P. umbilicalis* are also abundant in Na and K, macrominerals which in balance with Ca and Mg, help reduce high blood pressure (Cofrades et al., 2010). *H. elongata* also has a long history of safe use and acceptability in cooking (Garcia-Vaquero, Lopez-Alonso, & Hayes, 2017).

The incorporation of edible seaweeds through blending or ingredient substitution has been explored in only a minority of processed meat products (Jeon & Choi, 2012); Cofrades, López-López, Solas, Bravo, & Jiménez-Colmenero, 2008; López-López, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2009; Choi et al., 2016; Jiménez-Colmenero et al., 2010, despite the fact that it is a relatively simple process and provides an effective measure to enhance meat quality (nutritionally and technologically speaking). A study on beef patties formulated with *H. elongata* found that texture and mouth-feel were improved without losing sensory quality (Cox & Abu-Ghannam, 2013). However, overall very little attention has been paid to the use of edible seaweeds as ingredients in processed meat products, especially in relation to sensory implications.

Therefore the purpose of this study was to investigate the addition of common edible seaweeds (*H. elongata* (Sea Spaghetti), *U. pinnatifida* (Wakame), *P. umbilicalis* (Nori) and *P. palmata* (Dulse)) as a salt replacer on compositional, physicochemical, volatile and sensory characteristics of reformulated frankfurters throughout storage (shelf life).

2. Material and methods

2.1. Meat and seaweed material

Pork loin from the *longissimus dorsi* muscle (90–95% visual lean) was purchased along with pork fat from a local supplier (Staunton Foods Ltd., Timoleague, Co. Cork, Ireland). Pork's loin composition was 2.6% fat, 73.3% moisture, 1.7% ash, 0.03% sodium, 0.082% NaCl, 0.2% glucose, 3.71% N, 23.2% protein, 1.09% monounsaturated fatty acids, 0.41% polyunsaturated fatty acids, 0.93% saturated fatty acids. The meat and fat were vacuum-packed and stored at −18 °C until required for frankfurter production. Starch, farina (milled wheat), paprika, tomato powder, sodium chloride (NaCl), phosphate, sodium caseinate, sodium ascorbate, sodium nitrite and carmine were obtained from AllinAll Ingredients Ltd. (Dublin, Ireland). Frankfurter spice and artificial cellulose casings (26 mm) were purchased from Fispak Ltd. (Dublin, Ireland).

Edible seaweeds (*H. elongata*, *P. umbilicalis* and *P. palmata*) were supplied by Wild Irish Seaweed Ltd. (Co. Clare, Ireland) and *U. pinnatifida* was sourced from Algamar (Pontevedra, Spain). All seaweeds were harvested along the west coast of Ireland or the north coast of Spain (*U. pinnatifida*). Irish Seaweed seaweeds were air-dried then dehumidified at Wild Irish Seaweed Ltd. facilities (Caherush Point, Spanish Point, Co. Clare, Ireland). Algamar seaweeds were dried at low temperature (< 42 °C) at Polígono de Amoedo (Pazos de Borbén, Pontevedra, Spain). These seaweeds were chosen, because of their composition, potential health implications, abundance on Irish and Spanish coasts, relatively widespread consumption/acceptability, and suitability for use as ingredients in these processed meat products.

2.2. Sample preparation

Air-dried seaweeds were milled to a maximum size of 1 mm by means of a mechanical grinder. Minced samples were stored in a −20 °C freezer, for no longer than 15 days until their incorporation in the food product.

The formulation of control frankfurters were as follows: pork loin (65%), pork fat (19%), ice/water (10.15%), starch (0.92%), farina (0.92%), frankfurter spice (0.5%), paprika (0.5%), tomato powder (0.25%), NaCl (2%), phosphate (0.25%), sodium ascorbate (0.05%), sodium caseinate (0.35%), sodium nitrite (0.0075%) and carmine (0.005%). For the formulation of seaweed frankfurters, 1% of salt was replaced with 1% edible seaweed, and 4% fat was replaced by more pork loin. Two independent batches of frankfurters with edible seaweeds were produced, following the same procedure.

The frozen meat and fat were cut into pieces and allowed to defrost at room temperature until they reached 4 °C. The meat and fat were then minced to a particle size of 3 mm through a mincer (Talsabell S.A., Valencia, Spain). The meat was weighed according to the formulations shown in Table 1 and placed in a bowl chopper (Seydelmann KG, Aalen, Germany). The required salt, preservatives (starch, farina, phosphate, sodium caseinate) and 3/4 of the water needed were added and mixed at low speed for 1 min; followed by adding and mixing the fat, seasoning (frankfurter spice, paprika and tomato powder) and the seaweed (only in the reformulated frankfurters) for further 2 min at high speed (3000 rpm). The remaining water and ice were finally added and mixed at high speed for ~45 s, until a final temperature of 15 °C was reached. The frankfurter mix was then placed in a casing filler (Mainca, Barcelona, Spain) and stuffed into cellulose casings of 26 mm diameter, hand-

Table 1
Frankfurter formulation.

	Control	*Himanthalia elongata*	*Undaria pinnatifida*	*Porphyra umbilicalis*	*Palmaria palmata*
Ingredients (g/100 g)					
Pork loin	65.0	69.0	69.0	69.0	69.0
Pork fat	19.0	15.0	15.0	15.0	15.0
Seaweed	–	1.0	1.0	1.0	1.0
Water/Ice 50:50	10.25	10.25	10.25	10.25	10.25
Starch	0.92	0.92	0.92	0.92	0.92
Farina	0.92	0.92	0.92	0.92	0.92
Frankfurter spice	0.5	0.5	0.5	0.5	0.5
Paprika	0.5	0.5	0.5	0.5	0.5
Tomato powder	0.25	0.25	0.25	0.25	0.25
NaCl	2.0	1.0	1.0	1.0	1.0
Phosphate	0.25	0.25	0.25	0.25	0.25
Sodium caseinate	0.35	0.35	0.35	0.35	0.35
Sodium ascorbate	0.05	0.05	0.05	0.05	0.05
Sodium nitrite	0.0075	0.0075	0.0075	0.0075	0.0075
Carmine	0.005	0.005	0.005	0.005	0.005
Salt and fat levels (%)					
Salt	2.0	1.0	1.0	1.0	1.0
Fat	19.0	15.0	15.0	15.0	15.0

linked (12 cm in length). The frankfurters were cooked at 100 °C in an electric steam-convection oven (Zanussi. Professional, Italy) until the internal product temperature reached 74 °C, tested by a hand-held temperature probe (Testo 110, Lenzkirch, Germany). The temperature of the frankfurters was then held at 74 °C for 10 min. All samples were cooked at the same time, in duplicate, and segregated. Lastly, the frankfurters were allowed to cool down for 20 min, then vacuum sealed into polyamide/polyethylene (PA/PE) laminate plastic bags (water vapour transmission rate of 2.8 g/m^2.day at 23 °C and 85% relative humidity (RH), an O_2 and N transmission rate of 10 cm^3/m^2.day at 23 °C and 0% RH and a CO_2 transmission rate of 150 cm^3/m^2.day at 23 °C and 0% RH) and stored at 4 °C in a chill room. Frankfurters used for compositional, colour, texture and water holding capacity analysis, volatile analysis and sensory evaluation were stored for no longer than 10 days. Frankfurters used in the microbiological analysis and the lipid oxidation analysis were stored for 55 and 63 days, respectively.

2.3. Compositional analysis

A reference meat sample with known moisture and fat content was analysed before measuring samples, to verify all analysis were correct. Moisture and fat content of 4 g of frankfurter were determined using the CEMSMART (moisture) and SMART Trac (fat) systems (CEM GmbH, Kamp-Lintfort, Germany) (Bostian, Fish, Webb, & Arey, 1985), after having been homogenised using a Büchi Mixer B-400 (BÜCHI Labortechnik AG,Meierseggstrasse 40, Postfach, CH-9230 Flawil 1, Switzerland) and quickly transferred into a moisture proof bag to avoid evaporative loss. Both analyses were undertaken in duplicate. Moisture of 2 g of seaweed was determined by drying the sample in a preheated to 135 °C oven for 2 h (Association of Official Analysis Chemists International (AOAC), 1996). Fat content of 5 g of seaweed was determined using Foss Soxtec Avanti 2055 Manual system (Foss, Hillerød, Denmark). Protein content of frankfurters and seaweed was measured using the Kjeldahl method (Suhre, Corrao, Glover, & Malanoski, 1982). The homogenised sample was weighed accurately (0.5 g) into digestion tubes. Kjeldahl tablets (Thompson & Capper Ltd., Runcorn, Cheshire, UK) were added and the tubes placed into a heated digestion block (FODD, Tewwtor™ digestor, Hillerød, Denmark). The Kjeldahl distillation unit was a FOSS, Kjeltec™ 2100 (Hillerød, Denmark). The protein was calculated using a nitrogen factor of 6.25. All analysis was carried out in duplicate.

The ash content of frankfurters and seaweed was determined in duplicate by a muffle furnace (Nabertherm GmbH, Lilienthal, Germany) (Association of Official Analytical Chemists, 1923), which was pre-heated to 600 °C. Approximately 5 g of well blended sample was used for the ash analysis. The salt content of frankfurters and seaweed was carried out, in duplicate, by titration using silver nitrate. Silver nitrate solution was standardised against 0.100% sodium chloride solution (0.1 N). Approximately 2 g of blended samples were put into muffle furnace to create ash (as in '2.5. Ash content analysis'). Ash was washed into conical flask with 20 ml distilled water and 2 ml of the chromate indicator was added. The flasks were potentiometrically titrated with 0.1 N silver nitrate from a clear yellow to an opaque light orange (+255 mV), after cooling to room temperature. Blank titration was performed using 20 ml distilled water. Sodium content was estimated by a conversion factor (2.5 g of salt per 100 g is 1 g of sodium per 100 g).

2.4. Colour analysis

The frankfurter samples were allowed to stay at room temperature for 1 h before measurement (Table 2). Six readings were taken per sample. The cooked frankfurters were cut in half and the colour was measured according to the CIE L* a* b* colour system using a Minolta CR400 colorimeter (Minolta Camera Co. Ltd., Osaka, Japan) with an 11 mm-diameter aperture and D_{65} illuminant. Previous calibration was done by the CIE Lab colour space system using a white tile (C: Y = 93.6, x = 0.3130, y = 0.3193) (Minolta calibration plate). The three coordinates of CIE L* a* b* represent the lightness of the colour ($L^* = 0$ yields black and $L^* = 100$ indicates diffuse white), its position between red/magenta and green (a^*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b^*, negative values indicate blue and positive values indicate yellow). Delta E 2000 formula was used to measure the visible difference between two colours, in this case between Control and reformulated frankfurters' colour. ColorTools Excel add-in was used to apply the formula. A smaller Delta E value (ΔE^*) represents a closer colour match.

2.5. Texture analysis

Texture Profile Analysis (TPA) (Bourne, 1978) was used to characterize the texture of the product and was performed with a Texture Analyser XT3i (Stable Micro Systems, Surrey, UK). Three (10 mm × 10 mm) cylindrical slices of frankfurter were compressed to 40% of their original height with a 35 mm diameter cylindrical probe (SMSP/35 Compression plate) in two cycles. Force–time deformation curves were derived with a 25 kg load cell applied at a constant crosshead speed of 1.5 mm/s. Texture profile parameters included: hardness (N), maximum force required to compress the sample; adhesiveness (N × mm), area under the abscissa after the first compression; springiness (mm), ability of the sample to recover its original form after deforming force was removed; cohesiveness (dimensionless), extent to which the sample could be deformed prior to rupture; and chewiness (N × mm), work required to masticate the sample before swallowing. All analyses were performed in triplicate and at room temperature.

2.6. Water holding capacity analysis

The water holding capacity was measured using the method described by Lianji and Chen (Lianji & Chen, 1989). Ten grams of frankfurter were wrapped in a cheese-cloth and placed in 30 ml centrifuge tubes lined with cotton wool at the bottom of each tube. The samples were centrifuged at 15,000 *g* for 10 min at 4 °C. After centrifugation, the cheese-cloth was removed and samples were reweighed. Water holding capacity was calculated using the following formula: % Water holding capacity = ((1-(B-A)/(B*M))*100, where B is the weight of sample before centrifugation, A is the weight of the sample after centrifuging, and M is the moisture content of sample determined using CEM SMART Trac system (CEM GmbH, Kamp-Lintfort, Germany).

2.7. Microbiological analysis

To ensure that frankfurter samples were safe for human consumption prior to sensory analysis, their microbiological quality was assessed by total viable count (TVC), after 1, 15 and 55 days of storage. Frankfurters were weighed (10 g) and placed into a stomacher bag. An initial 10-fold dilution was performed by adding 90 ml of sterilised maximum recovery (MRD) diluent (Oxoid, Baingstoke, UK). Then, the sample was stomached (Steward Stomacher 400 Lab Blender, London, UK) for 3 min, and the homogenate was 10-fold serially diluted using MRD solution. For the enumeration of TVC, 1 ml of appropriately diluted sample was inoculated on duplicated dry-total count plates (20 cm^2) (Nissui Pharmaceutical, Co. Ltd., Japan), followed by incubation at 30 °C for 48 h. All analysis was undertaken in duplicate. The limit of microbiological acceptability was set at 5 log CFU/g (Food Safety Authority of Ireland, 2019).

2.8. Lipid oxidation analysis

The lipid oxidation of frankfurters was assessed after 63 days of storage by the thiobarbituric acid (TBA) assay (Siu & Draper, 1978).

Table 2
Composition of seaweeds used in the formulation and composition, colour, texture analysis, water holding capacity and lipid oxidation values (TBARS) of frankfurters.

	Control	*Himanthalia elongata*	*Undaria pinnatifida*	*Porphyra Umbilicalis*	*Palmaria palmata*	
	M ± SE	M ± SE	M ± SE	M ± SE	M ± SE	*P*-value
Composition						
Seaweeds						
% Moisture	N/A	16.31 ± 0.34^{a}	10.77 ± 0.03^{b}	8.99 ± 0.16^{c}	10.04 ± 0.41^{d}	< 0.001
% Fat	N/A	1.42 ± 0.14^{ab}	2.30 ± 0.11^{c}	1.13 ± 0.03^{a}	1.69 ± 0.14^{b}	< 0.001
% Protein	N/A	5.84 ± 0.23^{a}	9.02 ± 0.59^{b}	33.57 ± 1.14^{c}	19.96 ± 0.20^{d}	< 0.001
% Ash	N/A	30.06 ± 0.23^{a}	51.18 ± 0.08^{b}	17.24 ± 0.25^{c}	25.64 ± 0.79^{d}	< 0.001
% Salt	N/A	15.16 ± 0.25^{a}	36.77 ± 1.94^{b}	5.88 ± 0.13^{c}	16.34 ± 1.06^{a}	< 0.001
Frankfurters						
% Moisture	59.57 ± 0.41^{a}	61.41 ± 0.19^{a}	61.31 ± 0.47^{a}	61.11 ± 0.72^{a}	61.90 ± 0.35^{a}	0.036
% Fat	11.92 ± 0.31^{a}	9.79 ± 0.36^{b}	9.70 ± 0.25^{b}	9.82 ± 0.18^{b}	9.33 ± 0.24^{b}	0.003
% Protein	18.61 ± 1.05^{a}	18.86 ± 0.89^{a}	18.99 ± 0.21^{a}	19.95 ± 0.53^{a}	19.62 ± 0.62^{a}	0.119
% Ash	3.12 ± 0.17^{a}	2.50 ± 0.07^{b}	2.77 ± 0.15^{ab}	2.41 ± 0.06^{b}	2.44 ± 0.07^{b}	0.005
% Salt	2.13 ± 0.05^{a}	1.37 ± 0.07^{b}	1.49 ± 0.08^{b}	1.19 ± 0.10^{b}	1.31 ± 0.10^{b}	0.001
Sodium (g)	0.853	0.548	0.595	0.476	0.522	–
Colour						
L*	70.93 ± 0.84^{a}	69.13 ± 0.62^{ab}	67.54 ± 5.33^{b}	64.79 ± 1.88^{c}	67.52 ± 2.30^{b}	< 0.001
a*	10.16 ± 0.61^{a}	8.72 ± 0.54^{b}	5.33 ± 0.18^{c}	6.91 ± 0.48^{d}	8.01 ± 0.51^{e}	< 0.001
b*	19.15 ± 0.42^{ab}	19.52 ± 0.54^{a}	18.41 ± 0.21^{b}	17.02 ± 0.70^{c}	18.31 ± 059^{b}	< 0.001
ΔE*	N/A	2.014	5.355	5.677	3.265	–
Texture						
Hardness (N)	55.67 ± 3.98^{a}	44.29 ± 4.96^{b}	39.33 ± 2.26^{b}	41.85 ± 2.91^{b}	44.39 ± 5.51^{b}	0.003
Adhesiveness (N*mm)	−0.26 ± 0.10^{ab}	−0.23 ± 0.08^{ac}	−0.16 ± 0.08^{ad}	−0.10 ± 0.09^{cd}	−0.07 ± 0.04^{d}	0.005
Springiness (mm)	0.89 ± 0.02^{ab}	0.87 ± 0.02^{a}	0.90 ± 0.06^{ac}	0.92 ± 0.03^{bc}	0.92 ± 0.03^{bc}	0.009
Cohesiveness	0.77 ± 0.01^{a}	0.75 ± 0.01^{b}	0.77 ± 0.01^{ab}	0.77 ± 0.01^{a}	0.77 ± 0.01^{a}	0.005
Chewiness	38.53 ± 2.91^{a}	28.84 ± 3.23^{b}	26.85 ± 2.30^{b}	29.65 ± 1.83^{b}	31.44 ± 4.61^{ab}	0.006
Water holding capacity	88.03 ± 2.38^{a}	88.21 ± 2.25^{a}	86.73 ± 3.83^{a}	87.90 ± 2.87^{a}	87.62 ± 3.42^{a}	0.960
TBARS (mg MDA/kg meat) (day 63 storage)	0.459 ± 0.04^{a}	0.836 ± 0.07^{b}	0.515 ± 0.06^{a}	0.564 ± 0.08^{a}	0.531 ± 0.09^{a}	< 0.001

M, mean; SE, standard error; N/A, not applicable; $P < .05$ considered statistically significant; $^{a–e}$, means in the same row bearing different superscript letter denote statistical significant differences ($P < .05$).

Five grams frankfurter samples were weighed into a container together with 25 ml distilled water and homogenised for 2 min using an Ultra Turrax T25 homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen, Germany). After adding 25 ml of trichloroacetic acid (TCA) (10%), the mixture was shaken vigorously and centrifuged at 8000 *g* for 15 min at 4 °C, and was filtered through a Whatman No.1 filter paper (Sigma-Aldrich, Darmstadt, Germany). Four ml of sample and 1 ml of 0.06 M 2-TBA were added in screw cap test tubes. Assay blanks contained 2 ml distilled water and 2 ml 10% TCA and 1 ml of 0.06 M TBA reagent. Test tubes were then placed in an 80 °C water bath for 90 min. The tubes were cooled at room temperature and vortex mixed to de-gas the samples. The absorbance of the filtrate was measured using a spectrophotometer at 532 nm against assay blank. The malondialdehyde (MDA) content of the samples was calculated using an extinction coefficient of $1.56 \times 10^{5}\,cm^{-1}$. Results were expressed as TBA reactive substances (TBARS) in mg MDA/kg of meat. All analyses were undertaken in duplicate. The maximum acceptable limit was established at 1 mg MDA/kg meat (Warriss, 2010).

2.9. Sensory evaluation

Frankfurters were assessed by a 25 member panel in one session. All subjects were selected on the basis that they consumed and purchased frankfurters regularly. Sensory analysis was conducted in panel booths, which conform to International Standards (ISO, 2007) (International Organization for Standardization, 2007). Five samples, cut in 3 cm length cylindrical slices, were presented to the panellists. Panellists were required to rinse with water before tasting each sample. Sample presentation order was randomised to prevent any flavour carryover effects (MacFie, Bratchell, Greenhoff, & Vallis, 1989) and coded with a 3-digits code.

In the sensory acceptance test, assessors were asked to indicate their score on a continuous 10 cm line scale, ranging from 0 at the left to 10 at the right, for each frankfurter in duplicate. The scores were expressed in cm from the left baseline. The following attributes were evaluated: colour, liking of appearance, liking of aroma, liking of flavour, liking of texture and overall acceptability (hedonic attributes). After training, the assessors then participated in a Ranking Descriptive Analysis (RDA) (Richter, de Almeida, Prudencio, & de Toledo Benassi, 2010). They were asked to assess the intensity of sensory attributes, such as tenderness, juiciness, salt taste, meat flavour, off flavour and seaweed flavour, which were also measured on a continuous 10 cm line scale. All samples were again presented in duplicate at ambient temperature.

2.10. Extraction and volatile compounds analysis

2.10.1. Standard solutions

The internal standard (2-phenyl-D5-ethanol), the check standards (1-butanol, dimethyl disulfide, butyl acetate, cyclohexanone and benzaldehyde) and external standards (where available) were supplied by Sigma-Aldrich Ireland Ltd. (Arklow, Co. Wicklow, Ireland). Internal and External standard solutions were prepared at 0.1% (*v*/v) in methanol and stored at −18 °C until required, but never longer than 3 months. The check standards solution was prepared with 124 μl 1-butanol, 96 μl dimethyl disulfide, 114 μl butyl acetate, 106 μl cyclohexanone and 96 μl benzaldehyde in 100 ml methanol. The standard solution mix (internal + check standards) was prepared as 100 μl of each solution in 10 mL distilled water daily as required. 10 μl of the standard solution mix were injected in the TD tubes with a syringe.

2.10.2. Thermal desorption

Ten grams of finely diced frankfurter (100 ul of internal standard was added at 50 ppm) were placed in stainless steel chamber inert coated pot and cooked in a Markes International Ltd. Micro-Chamber/Thermal Extractor (Llantrisant, UK) for 40 min at 72 °C with a nitrogen flow of 50 ml/min. Samples were analysed in triplicate. Extraction of volatile compounds was carried out while the frankfurters were cooking. Volatiles were trapped in a TD Tenax Carbograph tube (Markes International Ltd) in series after a prepared sodium sulphate TD tube (Markes International Ltd) dry packed with 1.5 g of sodium sulphate granules which is used as a moisture trap. The flow rate through the tubes was monitored to ensure that flow was not interrupted during the cooking process due to condensation of moisture in the extraction tubes. Control and reformulated samples were treated in an identical manner.

2.10.3. Gas chromatography-mass spectrometry (GC-MS)

A Unity 2 Thermal Desorption Unit (Markes International Ltd) was used to concentrate the volatiles and remove any excess moisture prior to direct transfer to the GC-MS. The Unity 2 was operated with purge gas of nitrogen at 50 psi at a flow rate of 1.2 ml/min. Tubes were dry purged for 2 min using a 1:20 split. A two stage desorption was employed: the first stage desorption was performed at 150 °C and held for 5 min, the second stage desorption was performed at 300 °C and held for 5 min. A 1:10 split was used for final tube desorption. The trap used was a materials emissions trap and this was held at 30 °C during tube desorption with a gas flow of 50 ml/min. Prior to trap desorption, a 2 min pre-trap fire purge was performed with a 1:50 split. Trap desorption was performed at 30–300 °C at a rate of 24 °C/min and held for 5 min with 1:10 split.

The GCMS system was a Agilent 7890A GC with a Agilent 5977B MSD (Agilent Technologies Ltd., Cork, Ireland). The column used was a capillary DB 624 UI (60 m × 0.3 mm × 1.8 μm) (Agilent Technologies Ltd., Ireland) with helium as the carrier gas. The column temperature started at 40 °C held for 5 min, increased to 230 °C at 5 °C/min and held at 230 °C for 35 min. The total run time was 78 min. Injector temperature was set at 250 °C. Desorbed volatile compounds were injected in splitless mode with a consistent pressure of 23 psi. The mass spectra of volatile compounds were generated by a MS quadrupole detector with ionisation voltage of 70 eV, 3.32 scans/s and a scanning mass range of 35–350 amu. The ion source temperature was 220 °C and the interface temperature was set at 280 °C.

A set of check standards were run as part of each sample set and abundances were compared to known amounts to ensure that both the TD extraction and MS detection were within specification. Volatile compounds were identified by comparing their mass spectra to the NIST 2014 mass spectral library and an in-house library created using authentic external compounds with target and qualifier ions for each compound, using MassHunter Qualitative Analysis Software (Agilent Technologies, Palo Alto, CA, USA). Spectral deconvolution was also performed to allow the identification of untargeted and co-eluting compounds using Automatic Mass Spectral Deconvolution and Identification System (AMDIS) (http://chemdata.nist.gov/mass-spc/amdis/). Linear retention indices (LRI) were determined using the method by Van Den Dool and Kratz (van Den Dool & Dec. Kratz, 1963) and matched against peer reviewed publications (Corral, Salvador, Belloch, & Flores, 2015; Gallego, Gelabert, Roca, Perales, & Guardino, 2012; Gianelli, Olivares, & Flores, 2011). Positive identification was only attained once the mass spectral match reached > 80%, using external standards where possible and when LRI's were within 10% of referenced values. The results were expressed in abundance values.

2.11. Data analysis

The compositional data, sensory evaluation data (sensory acceptance test and RDA), the data acquired by instrumental methods and TBARS values were evaluated by Bonferroni multiple comparison analysis (one-way ANOVA), using SPSS Statistics software version 24 (IBM Corp., Armonk, NY), to determine if there were statistically significant differences ($P < .05$) between different formulations. In addition, sensory evaluation data and instrumental measurements were further analysed using ANOVA-Partial Least Squares Regression (APLSR). Regression coefficients were analysed by Jack-knifing to derive significance indicators for the relationships determined in the quantitative APLSR (data not shown). This further analysis was performed using the Unscrambler X Software, version 10.3 (CAMO ASA, Trondheim, Norway).

R software (RStudio, Inc., Boston, MA) for statistical computing and graphics was used to analyse the volatile component profile and obtain a hierarchical clustering heatmap with the abundance values of volatile compounds derived from each frankfurter formulation. Principal Component Analysis (PCA) was also carried out to aid the association of volatile compounds within the frankfurter samples.

3. Results and discussion

3.1. Physiochemical analysis

The moisture content of the reformulated samples was not statistically different from the control (Table 2). The moisture content of frankfurters was inversely associated with fat and salt content, in that the lower fat samples correlated to higher moisture, and higher salt samples correlated to lower moisture content. Similar findings were obtained by Tobin et al. on pork breakfast sausages (Tobin, O'Sullivan, Hamill, & Kerry, 2013). Lower moisture contents also correlated with lighter colour of frankfurters, and vice versa, matching results by Ahmed et al. (Ahmed, Miller, Lyon, Vaughters, & Reagan, 1990), where the colour in sausages was generally influenced by fat content and moisture. The water holding capacity of all frankfurter samples was at a relatively high level (> 86.0%), although no significant differences were recorded (Table 2). Water holding capacity is the ability of food to hold its own or added water during the application of force, pressure, centrifugation, or heating. It is also the ability of food to retain water against gravity and has shown to play a major role in the formation of food texture (Gyawali & Ibrahim, 2016). The water binding property of myoglobin is expected to decrease in meat products when salt content is reduced, because salt plays an important role in solubilising and improving functionality of myoglobin (Desmond, 2006). However this was not the case in this study. The salt contents of the seaweed preparations are also provided in Table 2. It is evident that the salt contents are significantly different ($P < .001$), with highest levels in the *U. pinnatifida* sample. These salts levels were quite different to those attained in a previous study (Pereira, 2011) and likely highlight differences in preparation. These seaweeds also naturally contain high levels of minerals, such as potassium, magnesium, zinc, iron and calcium that can all add to the "saltiness" perception and play a role in flavour development (Hotchkiss, 2010). The salt content of reformulated frankfurters was significantly reduced ($P < .001$) (Table 2). Salt reduction exceeded 30% for all products but was highest in the reformulated frankfurters containing red seaweeds (*P. umbilicalis* and *P. palmata*).

All the reformulated frankfurters has statistically significantly ($P < .05$) lower fat contents than the Control (Table 2). Although the fat contents in the reformulated frankfurters were not reduced enough to be classified as a reduced fat product, fat content reduction ranged from 17.6–21.7%. The fat content of each seaweed preparation is also provided in Table 2, and were similar to values obtained in a previous study (Pereira, 2011).

The two red seaweeds (*P. umbilicalis* and *P. palmata*) had a significantly higher levels ($P < .001$) of protein than the two brown seaweeds (*H. elongata* and *U. pinnatifida*), but this was not evident in the reformulated frankfurters (Table 2). The protein content in the reformulated frankfurters was statistically similar to the Control. Seaweed

protein contents are highly variable with a previous study finding protein contents ranging from 5 to 39% in *H. elongata*, *U. pinnatifida*, *P. umbilicalis* and *P. palmata* (Pereira, 2011). This same study also found variable ash contents in these same seaweeds from 12 to 40% (Pereira, 2011). The ash contents of the seaweed samples in this study varied from 17.24 to 51.18%, with highest levels in the *U. pinnatifida* preparation (Table 2). The ash content of the reformulated frankfurters was lower than that of the Control samples (Table 2), possibly due to the salt reduction. Similar findings were obtained by Fellendorf et al. (Fellendorf, O'Sullivan, & Kerry, 2017), who observed an increase in ash content with an increase of salt content in black pudding. Frankfurters reformulated with *U. pinnatifida* accounted a higher percentage of ash, while frankfurters reformulated with *P. umbilicalis* accounted the lowest percentage.

Significant colour ($P < .001$) differences were evident between Control and reformulated frankfurters (Table 2). L* values were found to be significantly ($P < .001$) lower (darker) in all the reformulated frankfurter samples except those containing *H. elongata*. The reformulated frankfurters also had significantly lower ($P < .001$) a* values (less magenta or more green) than the Control, and this was especially evident in the reformulated frankfurter with added *U. pinnatifida*. The b* values were also significantly lower ($P > .001$) in the reformulated frankfurters (more blue, less yellow), except for those with added *H. elongata*. However, previous studies have reported that the addition of 3.3% *H. elongata* reduced the L*, a* and b*, values in frankfurters and the incorporation of *H. elongata* (2.5%) and *U. pinnatifida* (2.5%) also reduced L*, a* and b* values in gel/emulsion systems (Cofrades, Lopez-Lopez, Solas, Bravo, & Jimenez-Colmenero, 2008); López-López et al., 2009). The addition of seaweeds to frankfurters, especially *P. umbilicalis* and *U. pinnatifida* impacted on the overall colour, as reflected by the ΔE* values (total colour difference) (Table 2). Changes in colour are likely directly due to the seaweed, but also possibly due to Maillard reactions, which are boosted in some pork products by the addition of seaweeds (Moroney, O'Grady, Lordan, Stanton, & Kerry, 2015).

The reformulated frankfurters had significantly lower hardness ($P > .003$) and chewiness ($P > .006$) values than the Control. López-López et al. (López-López et al., 2009) showed that a 5% addition of seaweed significantly increased the chewiness and hardness in reformulated frankfurters. The contradiction between these results is likely to relate to differences in seaweed preparation and the amount added. Cohesiveness was reduced ($P > .005$) in reformulated frankfurters with the added *H. elongata*. Springiness increased ($P > .009$) in reformulated frankfurters with added *P. umbilicalis* or *P. palmate* and adhesiveness statistically increased ($P > .005$) in the reformulated frankfurters with added *P. umbilicalis* or *P. palmata* (Table 2).

3.2. Microbiological analysis

The microbiological quality of all the frankfurters at day 1 of storage was below 10 CFU/g (Fig. 1) and remained below the limit of acceptability (Log 5 CFU/g) at 15 days of storage. However the reformulated frankfurter samples containing *U. pinnatifida* had a higher TVC than all other samples ($P = .027$) at day 15. The limit of acceptability (> 5 CFU/g) was exceeded in all frankfurter samples after 55 days of storage, and again the reformulated frankfurter samples with *U. pinnatifida* had significantly higher TVC than other samples ($P < .001$). Therefore, the shelf life from in the reformulated frankfurter samples with added 1% *U. pinnatifida* is less than all other samples, and these results are in agreement with the microbiological analysis carried out by López-López et al. (López-López et al., 2010) in beef patties. The estimated shelf-life of the control samples was 50 days, and it was similar to the shelf-life of frankfurter samples in a study by O'Neill et al. (O'Neill, Cruz-Romero, Duffy, & Kerry, 2018), in which the same control formulation was used. Salt has a preservative effect in processed meat by lowering water activity, which helps control microbial growth.

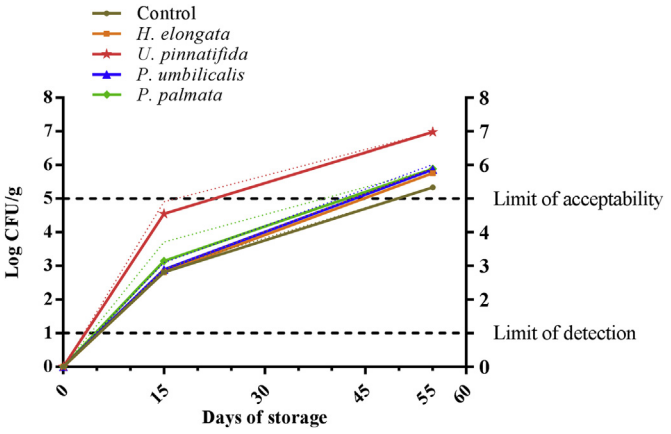


Fig. 1. Total viable count changes across storage period of frankfurters. Dot lines represent standard deviations.

The reduction thereof can then have a negative impact on the shelf-life. However, the estimated shelf-life of reformulated frankfurters with added *H. elongata*, *P. umbilicalis* or *P. palmata* was similar to the Control.

3.3. Lipid oxidation

The TBA assay for lipid oxidation generated TBARS values (Table 2) were below the maximum acceptable limit of 1 mg MDA/kg meat for all samples, at day 63 of storage. The frankfurter samples formulated with *H. elongata* had significantly ($P < .05$) higher TBARS values which may indicate a greater susceptibility to lipid oxidation or reduced antioxidants in comparison to the other samples. Seaweeds can reduce lipid oxidation due to their antioxidant content but may also affect the susceptibility to lipid oxidation due to their polyunsaturated fatty acids content. The addition of brown seaweed polysaccharides was demonstrated to reduce lipid oxidation in pork patties during cooking, owing to the formation of potential antioxidant melanoidins (Maillard reaction products) (Moroney et al., 2015). The addition of 40% *H. elongata* powder to beef patties also exhibited a high antioxidant capacity, and yet such high levels of addition were deemed acceptable in terms of sensory quality (Cox & Abu-Ghannam, 2013). We did not find a direct comparable study measuring lipid oxidation in frankfurters formulated with seaweed; however, a study by Francisco Jiménez-Colmenero et al. (Jiménez-Colmenero, 2007) found that if olive oil was used to partially replaced pork back fat in fermented sausages and frankfurters, no oxidation issues were detected. We can conclude that the rate and extent of lipid oxidation in the reformulated frankfurters in this study is not limiting shelf life.

3.4. Sensory consumer evaluation

Sensory evaluation and instrumental data are presented in the PLSR plot in Fig. 2 with the corresponding scores and ANOVA *P*-values in Tables 2 and 3.

3.4.1. Sensory and hedonic attributes

P. umbilicalis and *P. palmata* frankfurters in particular are highly positively correlated with each other in terms of sensory properties, while *U. pinnatifida* frankfurters were somewhat also correlated. Both *P. umbilicalis* and *P. palmata* frankfurters are negatively correlated in comparison to the Control (Fig. 2).

In a study by Tobin et al. (Tobin, O'Sullivan, Hamill, & Kerry, 2012a), frankfurters with salt contents lower than 1.5% had a significant negative impact ($P < .05$) on consumer acceptance. However, in a subsequent study by Fellendorf et al. (Fellendorf, O'Sullivan, & Kerry, 2015) white pudding products containing 0.6% sodium were accepted ($P < .05$) without using ingredient replacers. All of the

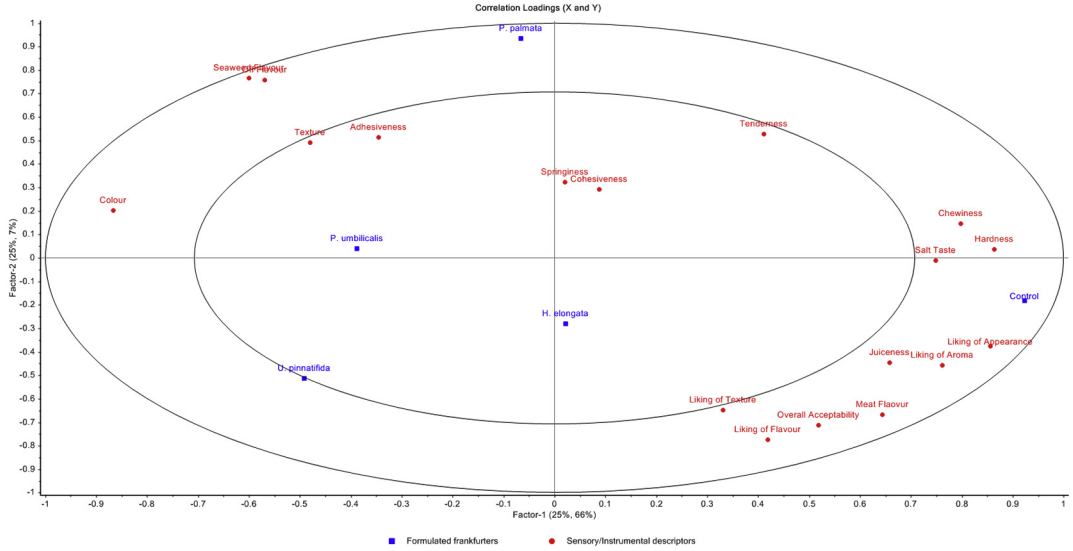


Fig. 2. Partial Least Squares Regression (PLSR) correlation loadings plot for frankfurter formulations with different salt replacers (Dulse = *Palmaria palmata*; Nori = *Porphyra umbilicalis*; Wakame = *Undaria pinnatifida*; S.Spaghetti = *Himanthalia elongata*). Shown are the X- (sensory and instrumental data) and Y- (treatment groups) variables for the first 2 PCs for the • (red) = sensory descriptors and instrumental variables, and ■ (blue) = control and reformulated frankfurters. The concentric circles represent 100% (outer) and 50% (inner) explained variance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

seaweeds assessed in this study changed the sensory profile of frankfurters. Some of those changes were deemed positive and others mainly negative by sensory panellists. Frankfurters containing *H. elongata* were the most accepted and closest to the Control (Table 3). The overall acceptability was mainly influenced by the seaweed flavour rather than by a reduction of salt content, as also evident in the study by Fellendorf et al. (Fellendorf, O'Sullivan, & Kerry, 2016) where the impact of seaweed-based ingredients was perceived negatively ($P < .001$) on white pudding flavour. Liking of appearance and liking of aroma of the Control samples were rated significantly higher than all other reformulated frankfurter samples (Table 3), highlighting the negative impact of seaweed addition on the appearance and aroma perceptions. This negative impact was lowest in the *H. elongata* sample (Fig. 2). Both liking of flavour and liking of texture followed a similar trend to liking of appearance and liking of aroma, except that reformulated samples with *H. elongata* were not rated significantly lower than the Control. Sensory evaluation scores were lowest in reformulated frankfurters with added *P. palmata* (Table 3).

Consumers perceived that the reformulated frankfurters were darker in colour than the control in the order of *P. umbilicalis* > *U. pinnatifida* > *P. palmata* > *H. elongata* (Tables 2 and 3). Those consumer perceptions were confirmed by the ΔE* values (Table 2), which showed a bigger difference in colour between the Control and the frankfurters with added *U. pinnatifida*, *P. umbilicalis* and *P. palmata*, than with those containing *H. elongata*. Colour intensity was strongly related to liking of appearance, the samples with a lower rating of appearance had a higher colour intensity, which in turn adversely impacted on the overall acceptability.

Texture (fine to coarse), tenderness (tender to tough) and juiciness (dry to juicy) are very important texture attributes. A significantly negative ($P > .01$) correlation between tenderness and *U. pinnatifida* and a significantly positive ($P > .01$) correlation between tenderness and *P. palmata* was observed (Fig. 2). This was also reflected in sensory evaluation scores in Table 3. Overall seaweed addition generally increased the coarseness and slightly increased the tenderness of the reformulated frankfurters. Frankfurters containing *P. umbilicalis* and *P. palmata* had lower sensory scores (Table 3) and were significantly ($P < .01$) negatively correlated to juiciness. Fat is considered essential

Table 3
Sensory evaluation scores for the different frankfurter formulations with edible seaweeds.

Attribute	Control M ± SE	*Himanthalia elongata* M ± SE	*Undaria pinnatifida* M ± SE	*Porphyra Umbilicalis* M ± SE	*Palmaria palmata* M ± SE	*P*-value
Sensory acceptance test (hedonic attributes)						
Overall acceptability	6.27 ± 1.63^{a}	5.78 ± 1.33^{ab}	5.17 ± 1.69^{b}	5.11 ± 1.66^{b}	3.90 ± 2.03^{c}	< 0.001
Liking of appearance	6.43 ± 1.94^{a}	5.37 ± 1.43^{b}	4.91 ± 2.13^{bc}	4.23 ± 2.31^{d}	4.57 ± 1.84^{cd}	< 0.001
Liking of aroma	5.57 ± 1.77^{a}	4.63 ± 1.81^{b}	4.39 ± 1.89^{b}	4.31 ± 1.85^{b}	3.95 ± 1.84^{b}	< 0.001
Liking of flavour	6.47 ± 1.55^{a}	5.99 ± 1.33^{a}	5.44 ± 1.88^{a}	5.40 ± 1.85^{a}	3.63 ± 2.46^{b}	< 0.001
Liking of texture	5.74 ± 2.34^{a}	5.64 ± 1.54^{a}	5.45 ± 1.72^{ab}	5.03 ± 1.94^{ab}	4.52 ± 1.^{97b}	0.014
Colour	2.82 ± 1.52^{a}	4.24 ± 1.38^{b}	5.90 ± 2.11^{c}	7.54 ± 1.09^{d}	5.61 ± 1.63^{c}	< 0.001
Ranking Descriptive Analysis (sensory attributes)						
Tenderness	4.86 ± 2.30^{a}	4.13 ± 1.84^{ab}	3.90 ± 1.80^{b}	4.69 ± 1.95^{ab}	4.84 ± 1.93^{a}	0.006
Juiciness	4.99 ± 2.00^{a}	4.46 ± 1.84^{ab}	4.12 ± 1.95^{ab}	3.97 ± 2.02^{b}	3.75 ± 1.98^{b}	0.003
Salt taste	5.76 ± 2.17^{a}	4.02 ± 2.03^{b}	4.43 ± 2.25^{b}	4.34 ± 2.02^{b}	4.55 ± 2.26^{b}	< 0.001
Meat flavour	6.13 ± 1.95^{a}	4.90 ± 1.69^{b}	4.73 ± 1.77^{b}	4.62 ± 1.97^{b}	3.48 ± 2.03^{c}	< 0.001
Seaweed flavour	0.68 ± 0.92^{a}	3.19 ± 2.26^{b}	3.01 ± 2.31^{b}	4.36 ± 2.65^{c}	6.52 ± 2.47^{d}	< 0.001
Off flavour	1.11 ± 1.67^{a}	2.16 ± 1.76^{b}	2.56 ± 2.36^{b}	2.95 ± 2.51^{b}	4.46 ± 2.89^{c}	< 0.001
Texture	3.85 ± 2.53^{a}	4.65 ± 1.80^{ab}	4.14 ± 2.00^{a}	5.75 ± 2.21^{c}	5.22 ± 2.19^{bc}	< 0.001

M, mean; SE, standard error. $P < .05$ considered statistically significant; $^{a–d}$, means in the same row bearing different superscript letter denote statistical significant differences ($P < .05$). Hedonic attributes were scored on a 10 cm-line liking scale and sensory attributes were scored on a 10 cm-line intensity scale.

in providing juiciness and hardness in meat products, as well as forming stable emulsions, reducing cooking loss, improving water holding capacity and binding properties (Choi et al., 2010). Sausages with a higher fat content are perceived juicier and easier to chew, but have less intense colour and are less shiny, compared to those with a lower fat content (Ventanas, Puolanne, & Tuorila, 2010). Similar findings on the effect of fat levels on sensory scores were found for low fat frankfurters (Choi et al., 2010); and for patties (Mohammad Nisar, Chatli, Sharma, & Sahoo, 2010; Serdaroglu, 2006; Tobin, O'Sullivan, Hamill, & Kerry, 2012b). Ruusunen et al. (Ruusunen et al., 2003) also found that higher salt content frankfurters resulted in increased firmness and juiciness, similar to results of this study. The correlations between juiciness and higher salt levels are most likely due to the greater extraction of myofibrillar proteins, thereby leading to enhanced moisture binding. On the contrary, Ventanas et al. (Ventanas et al., 2010) found sausages with a higher salt content were perceived as less juicy, harder to chew and having a more intense colour than those with a lower salt content.

Additionally the regression coefficient *P*-values (not shown) demonstrated a negative correlation between all four reformulated frankfurters and salt taste, with the latter statistically significant for *P. umbilicalis* ($P < .01$) and *P. palmata* ($P < .001$). Frankfurters with added *H. elongata* scored lowest (Table 3). Salt taste is directly correlated to salt content; therefore, as expected, the salt taste of Control samples scored significantly higher than the reformulated samples. No significant difference in salt content was found between the reformulated frankfurters. The flavour profile of frankfurters is highly influenced by salt taste and meat flavour (Ruusunen, Simolin, & Puolanne, 2001). In this study, meat flavour was negatively correlated to frankfurters containing *P. umbilicalis* and *P. palmata*. However, both frankfurters containing *U. pinnatifida* or *H. elongata* had positive ($P < .01$) correlations to meat flavour perception (Fig. 2). On the other hand, the meat flavour of the Control samples scored significantly higher than the reformulated samples. This result is in agreement with Tobin et al. (Tobin et al., 2012a), who concluded that meat flavour in frankfurters is positively related to fat content. The positive correlation between the higher fat content and the perceived meat flavour by consumers may be explained by the fact that fat is a flavour enhancer and thus contributes to the overall flavour (Ruusunen et al., 2001). Fat also interacts with other ingredients and helps to develop texture, mouth feel and provides a lubricating effect in processed meats (O'Sullivan, 2016).

The diversity of volatiles compounds present in seaweeds produce different odours which contribute to the 'seaweed flavour' attribute in this study. Characteristic seaweed flavour was only detected in the reformulated frankfurters, which influenced overall acceptability of these products. The inclusion of *U. pinnatifida*, *P. umbilicalis* and *P. palmata* had a significant ($P < .001$) impact on the seaweed flavour of the reformulated frankfurters (Fig. 2). Reformulated frankfurters containing *P. palmata* or *P. umbilicalis* were found to have the strongest seaweed flavour, followed those formulated with *H. elongata* and *U. pinnatifida* (Table 3). Seaweed flavour was also found to be a factor that reduced the acceptability of the reformulated frankfurters (Jiménez-Colmenero et al., 2010; López-López et al., 2009). An intense seaweed flavour is a rather unfamiliar flavour to Western consumers. In any event, the unfamiliar flavour intensity to western cultures can always be limited by selecting less strongly flavoured seaweed or reformulating via selected seasoning. Even so, for population sectors to whom seaweed flavour is familiar, the characteristic flavour of frankfurter formulated with seaweed would not be an obstacle. Seaweed flavour was also an important factor that contributed to the perception of off-flavour. Reformulated frankfurters scored significantly higher than Control the samples for off-flavour. Off-flavour was negatively correlated to frankfurters containing *H. elongata* and *U. pinnatifida* and positively and significantly correlated to frankfurters with *P. umbilicalis* ($P < .001$) and *P. palmata* ($P < .001$) (Fig. 2). *P. palmata* frankfurters were percieved to have the highest off-flavour (Table 3) and thus not accepted by consumers. Similarly, in a study by Jimenez-Colmenero et al. (Jiménez-Colmenero et al., 2010) panellists were able to detect the addition of *H. elongata* seaweed as an off-flavour (seaweed-like).

3.4.2. Sensory texture profile analysis

The Control frankfurters significantly differed ($P < .001$) from reformulated frankfurters in terms of hardness and chewiness, except for those with added *P. palmata* (Table 3). Controls also significantly differed ($P < .001$) from *H. elongata*-containing frankfurters for cohesiveness (Table 3). Adhesiveness was statistically significantly different ($P < .001$) between the Control and those with added *P. umbilicalis* or *P. palmata* (Table 3). Springiness was statistically different ($P < .001$) between the reformulated frankfurters with added *H. elongata*, *P.umbilicalis* or *P. palmata* (Table 3).

3.5. Volatile compounds

A total of 22 terpenes, 21 alcohols, 17 aldehydes, 8 esters, 11 ketones, 4 phenylpropenoids, 4 sulphur compounds, 3 phenols, 1 pyrazine, 1 furan, 1 benzene and 1 pyrrole were detected in the Control and reformulated frankfurters. Quite a lot of diversity existed between the volatile profiles, especially in relation to levels of abundance of individual volatiles. Terpenes and esters attained their highest levels in the reformulated frankfurters containing *H. elongata*; Ketones, phenylpropanes and phenols the most abundant volatile classes in reformulated frankfurters containing *P. umbilicalis*. Alcohols, aldehydes and sulphur compounds were at greatest abundance in reformulated frankfurters containing *P. palmata*; while the reformulated frankfurters containing *U. pinnatifida* did not have any predominant class of volatile compounds. High amounts of o-cymene, ß-phellandrene, 4-methyl-2-pentanol, γ-terpinene, α-thujene, 3-carene, β- pinene and α-pinene were present across all samples, while low levels of 2-undecenal, pyrrole, methyl valerate and ethyl ether were also present in all samples. 4-Thujanol; (*Z*)-p-mentha-2,8-dien-1-ol; alpha-terpinyl acetate; methional; pyrrole; octanal; propanal, 2-methyl and 2-butanone were at lower levels in all four reformulated frankfurters compared to Control. It is apparent from the heatmap (Fig. 3) that the reformulation of frankfurters influenced the abundance of volatile compounds. α- and ß-pinene (terpene), prenol (phenol), 2-ethyl-1-hexanol (alcohol), butanal and propanal (aldehydes) and dimethyl trisulfide (sulphur compound) were strongly correlated to reformulated frankfurters with *H. elongata* (Fig. 2). Elemicin (phenylpropane), pentanal and hexanal (aldehydes), 3-heptanol and 1-butanol (alcohols), methyl isobutyl ketone (ketone) were strongly correlated to reformulated frankfurters with *P. umbilicalis*. Isopropyl alcohol and 1-penten-3-ol (alcohols) and α-thujene and α-cubebene (ketones) were strongly correlated to reformulated frankfurters with *P. palmata*; while reformulated frankfurtes with *U. pinnatifida* was strongly correlated to ß-bisabolene (terpene), benzene, 4-methyl-2-pentanol and 1-octen-3-ol (alcohol), (Z)-2-hepatanal and benzeneacetaldehyde (aldehydes) (Fig. 2).

The characteristic volatile compounds of some edible seaweeds have been analysed by GC–MS, which found that their volatile profile depends upon the species, its geographical origin, processing method and environmental conditions (Watanabe et al., 2014). Some studies found that brown seaweeds produce sulphur compounds, C_{11}-hydrocarbons and diterpenes while red seaweeds are known to produce more halogenated compounds containing bromine and iodine, which contribute to a sweet note in the aroma of this specie of seaweeds (Leite Santos & Narain, 2018). Similar findings were obtained in this study, with terpenes having the highest abundance in the reformulated frankfurters containing the brown seaweeds (*H. elongata* and *U. pinnatifida*). On the contrary, no specific halogen compounds were found in the reformulated frankfurters containing red seaweeds.

In terms of volatile profiles, all reformulated frankfurters in batch 1 differed from the reformulated frankfurters in batch 2, highlighting that volatile profiles are more easily impacted by small changes in

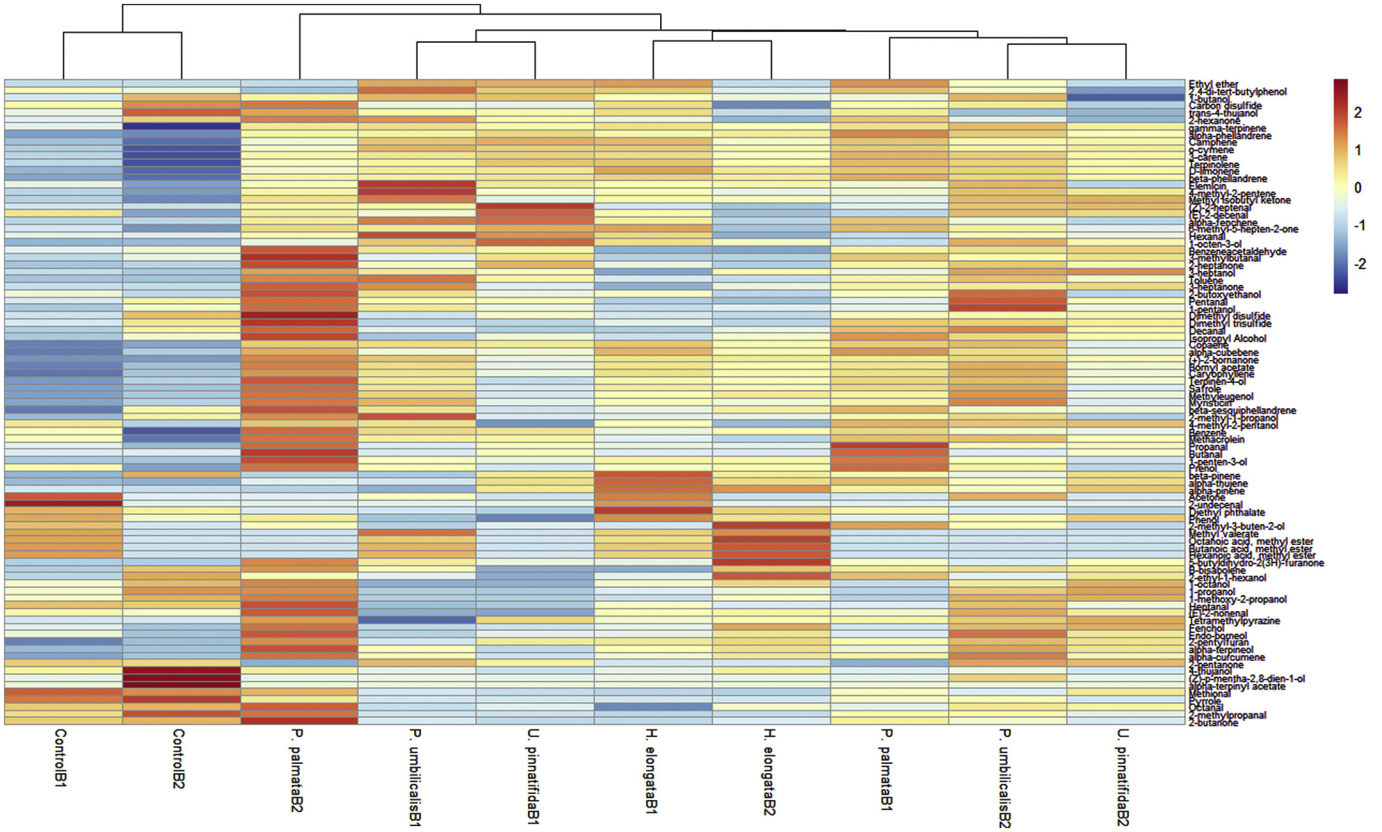


Fig. 3. Hierarchical clustering heatmap of volatile compounds derived from frankfurters produced with different edible seaweeds in two batches. The abundance of the 50 most contributing volatile compounds is reflected in a degraded scale of colour: from red (higher abundance) to blue (lower abundance). B1, batch 1; B2, batch 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

composition and processing in products such as frankfurters with multiple ingredients and extensive processing. The volatile profiles of *P. palmata* reformulated frankfurters differed most between batches (Fig. 3). None of the other analysis undertaken, showed evidence of batch variation.

The volatile compounds making the greatest contribution to differences between samples were o-cymene, camphene, 3-carene, β-phellandrene, terpinolene, 2-methyl-propanal, D-limonene, copanene, (+)-2-bornanone, bornyl acetate, terpinen-4-ol, caryophyllene, safrole and alpha-curcumene (mostly terpenes) (Fig. 4). It is likely that many of these compounds are directly related to the seaweeds as the volatile profile of seaweeds can be very diverse (Maruti, Durán-Guerrero, Barroso, & Castro, 2018). Therefore, due to the strong marine flavour profile of seaweeds, it is difficult to determine whether changes in the volatile profile are due to a modification of the flavour profile through the addition of characters that are typical of seaweeds (e.g. marine, seaweed, salty, sulphur) or to a modification of the flavour profile through enhancement or potentiation of flavour characters typical of the frankfurters recipe to which the seaweed has been added.

4. Conclusion

The inclusion of seaweed and reduction of salt and fat impacted on the sensory and physiochemical properties of frankfurters. Significant differences in colour, liking of appearance, aroma, flavour and texture attributes were evident between the samples. The overall acceptability of reformulated frankfurters containing seaweed was greatly influenced by the type of added seaweed, with reformulated frankfurters containing *H. elongata* being the most accepted. Inclusion of seaweed greatly influenced the abundance of volatiles compounds, but further research is required to determine the impact of individual volatile compounds on the sensory properties.

The addition of *U. pinnatifida*, *P. umbilicalis*, *P. palmata* and especially *H. elongata* have the potential to improve the nutritional quality through mainly salt reduction. It may require further processing of seaweed prior to addition, optimization of dose rates, combinations of seaweeds and/or highlighting potential nutritional benefits before such products are accepted by consumers unfamiliar with seaweeds in their diet.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2019.108001.

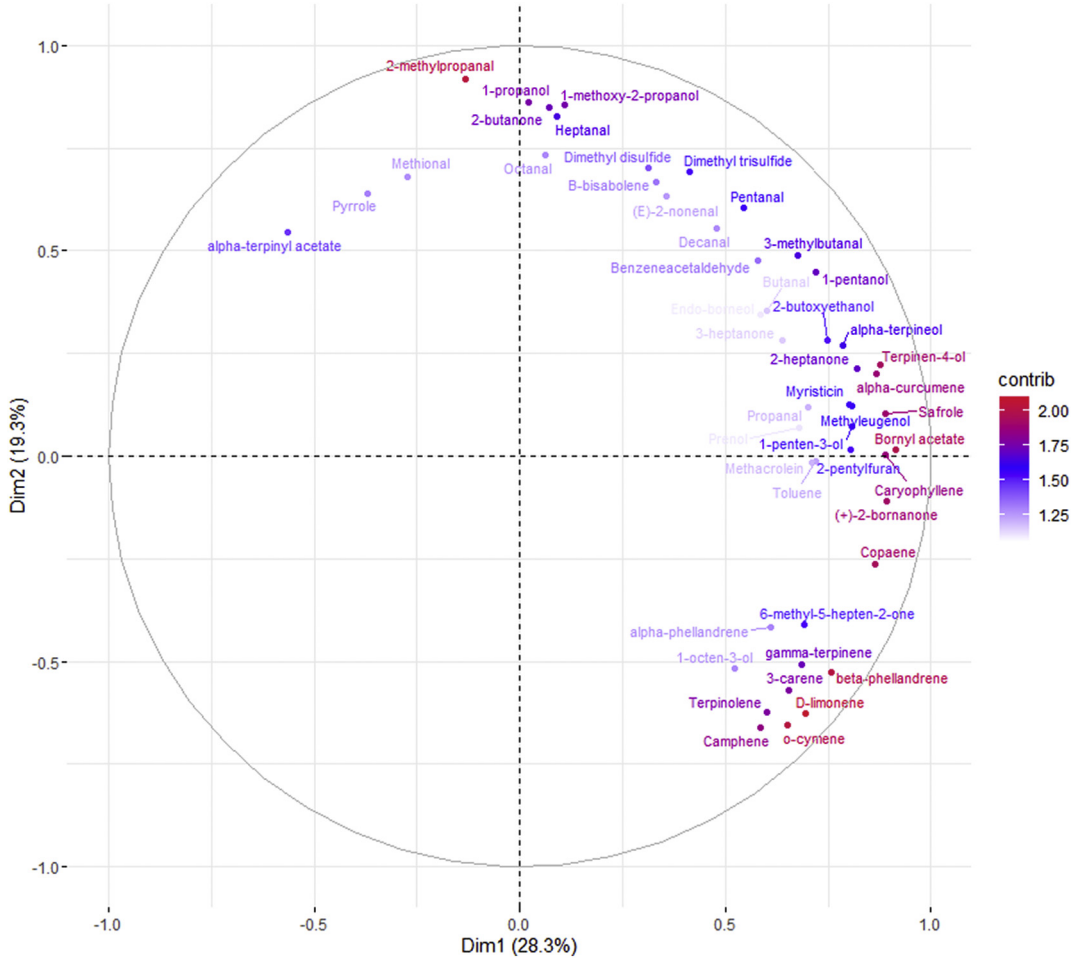


Fig. 4. Principal Component Analysis (PCA) plot for the volatile compounds making the highest contribution to the differences between the frankfurters reformulated with different salt replacers (Dulse = *Palmaria palmata*; Nori = *Porphyra umbilicalis*; Wakame = *Undaria pinnatifida*; S.Spaghetti = *Himanthalia elongata*) and controls. The contribution of the 50 most determining volatile compounds is reflected in a degraded scale of colour: from red (higher contribution) to fade purple (lower contribution). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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