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IRISH SEAWEED SPECIES IN PROCESSED MEAT PRODUCTS (SAUSAGES): CHARACTERISATION, FAT AND SALT REDUCTION, AND SENSORY ACCEPTABILITY STUDIES

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, either at 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.

Certified by:



Halimah O. Mohammed

April 2024

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This thesis is dedicated to my daughter, Imani Olatide Baruwa.

Abstract

Consumers are becoming more aware of the negative impacts of excessive fat and salt consumption on their overall health. One of the primary food groups contributing to this is processed meat products, such as sausages. The primary objective of this thesis was to assess the use of Irish brown (sea spaghetti (SS) and Irish wakame (IW)) and red (dulse (PP) and nori) seaweed species as potential reducers/replacers of fat and salt in sausages using sensory (hedonic and intensity) and physicochemical indicators. A survey on attitudes towards seaweeds and seaweed-food products indicated most Irish consumers trusted new foods and had consumed either seaweed or seaweed-containing products, which indicated a positive reaction towards the consumption of new seaweed food products. Seaweeds were initially characterised based on their nutritional, bioactive, thermogravimetric and technological properties. Seaweed species in this study represented potential nutritional and dietary sources of protein, amino acids, dietary fibre, minerals and health-promoting bioactive antioxidant compounds. Subsequently, these seaweed species were incorporated into pork sausages at 1.0%, 2.5% and 5.0% to determine acceptable inclusion levels from a sensory perspective. PP's maximum acceptable inclusion level was 1.0%, and 2.5% was for SS, IW, and nori. These seaweeds were further incorporated into reduced-fat (20%, 15% and 10%) and -salt (1.5%, 1.0% and 0.5%) pork sausages at selected maximum levels. The physicochemical and sensory characteristics of reformulated sausages were analysed. Subsequently, shelf-life evaluation (lipid oxidation and microbiological analysis) was performed on selected formulations stored under aerobic (AP), MAP70/30 (70% N2:30% CO2), MAP80/20 (80% O2:20% CO2) and vacuum (VP) conditions. Based on sensory results, sausage

formulations were selected: SS10f/0.5s, IW15f/1s, PP10f/0.5s and nori10f/1.0s. MAP70/30 and VP were the most effective packaging systems in controlling lipid oxidation and microbial growth. A consumer acceptability study comparing optimised seaweed reduced-fat and -salt sausages against experimental (EC) and commercial (CC) controls was carried out, and results clearly showed that consumers preferred control sausage samples to reduced-fat and -salt seaweed-containing sausages. An odour-induced saltiness enhancement (OISE) strategy employing bacon stimuli (0.08%) was then assessed to determine if this approach might improve the acceptability of the least liked consumer seaweed sausage formulation (nori10f/1.0s). The bacon stimuli did not enhance the saltiness perception of nori10f/1.0s, but interestingly, it positively influenced the saltiness flavour of reduced-fat and -salt sausages (C2B).

Publications and Presentations

Publications

Mohammed, H.O., O'Grady, M.N., O'Sullivan, M.G., Hamill, R.M., Kilcawley, K.N. and Kerry, J.P. (2021). An Assessment of Selected Nutritional, Bioactive, Thermal and Technological Properties of Brown and Red Irish Seaweed Species. *Foods*, *10*(11), 2784.

Mohammed, H.O., O'Grady, M.N., O'Sullivan, M.G., Hamill, R.M., Kilcawley, K.N. and Kerry, J.P. (2022). Acceptable Inclusion Levels for Selected Brown and Red Irish Seaweed Species in Sausages. *Foods*, *11*(10), 1522.

Manuscripts in preparation

Mohammed, H.O., O'Sullivan, M.G., Hamill, R.M., Kilcawley, K.N. and Kerry, J.P. Irish consumers' attitudes towards seaweed and seaweed-food products (In preparation for submission to *Irish Journal of Agricultural and Food Research).*

Mohammed, H.O., O'Sullivan, M.G., Hamill, R.M., Kilcawley, K.N. and Kerry, J.P. Utilisation of brown Irish seaweed species (sea spaghetti and Irish wakame) as partial fat and salt replacers in pork sausages: physicochemical, sensorial and shelf-life characteristics (In preparation for submission to *Journal of Meat Science*).

Mohammed, H.O., O'Sullivan, M.G., Hamill, R.M., Kilcawley, K.N. and Kerry, J.P. Physicochemical, sensorial and shelf-life properties of pork sausages containing red

Irish seaweed species (dulse and nori) as partial fat and salt replacers (In preparation for submission to *Journal of Food Packaging and Shelf Life*).

Mohammed, H.O., O'Sullivan, M.G., Hamill, R.M., Kilcawley, K.N. and Kerry, J.P. Irish consumers' attitudes towards seaweed-containing sausages: using seaweed as potential ingredients in salt and fat reduction strategies (In preparation for submission to *Journal of Sensory Studies).*

Mohammed, H.O., O'Sullivan, M.G., Hamill, R.M., Kilcawley, K.N. and Kerry, J.P. Can odour induced salt enhancement (OISE) improve the sensory acceptability of reformulated pork sausages containing red Irish seaweed nori (*Porphyra umbilicalis*)? (In preparation for submission to *Journal of Sensory Studies*).

Poster presentations

Halimah O. Mohammed, Michael N. O'Grady, Kieran N. Kilcawley, Ruth M. Hamill, Maurice G. O'Sullivan, Joseph P. Kerry. Characterisation of the nutritional and bioactive properties of brown and red Irish seaweeds for potential use as functional ingredients in processed meat products. In: *Proceedings of the 47th Annual Food Science and Technology Conference*, Cork, Ireland. 6th- 7th of December, 2018.

Halimah O. Mohammed, Kieran N. Kilcawley, Ruth M. Hamill, Joseph P. Kerry, Maurice G. O'Sullivan. Acceptable inclusion levels for selected red and brown Irish seaweed species in pork sausages. In: *Proceedings of the 65th International Congress of Meat Science and Technology*, Potsdam/Berlin, Germany. 4th- 9th August, 2019.

Halimah O. Mohammed, Maurice G. O'Sullivan, Kieran N. Kilcawley, Ruth M. Hamill, Joseph P. Kerry. Effects of Red seaweed species on Physicochemical and Sensory properties of low-salt, low-fat sausages. In: *Proceedings of the 48th Annual Food Science and Technology Conference,* Limerick, Ireland. 16th December, 2019.

Oral presentation

Halimah O. Mohammed, Maurice G. O'Sullivan, Joseph P. Kerry. Reduction of salt and fat in processed meats (sausages) using edible seaweed species. Oral presentation at School of Food and Nutritional Sciences Internal conference, University college cork, Cork, Ireland. 13th December, 2019.

Abbreviations

a*- Redness

ANOVA - Analysis of variance

AP- aerobic packaging

b*- Yellowness

CC- Commercial control

CIE - Commission Internationale de l´Eclairage

CVDs- cardiovascular diseases

C1- control

C2- reduced fat and salt control

C2N- C2+ nori seaweed

C2B- C2 + bacon stimuli

C2NB- C2 + nori seaweed + bacon stimuli

DPPH- 2,2-Diphenyl-1-picrylhydrazyl

DW- Dry weight

EC- Experimental control

FRAP- Ferric reducing antioxidant power

FSAI - Food Safety Authority Ireland

IW- Irish wakame

IW15f/1s- Irish wakame sausage formulation containing 15% fat and 1% salt

L* - Lightness

MAP70/30- Modified atmosphere packaging containing 70% N_2 and 30% CO_2

MAP80/20- Modified atmosphere packaging containing 80% O_2 and 20% CO_2

MUFA- Monounsaturated fatty acid

NaCl- Sodium chloride

Nori10f/1.0s- nori sausage formulation containing 10% fat and 1% salt

ODP- Optimised descriptive profiling

pH- potential of hydrogen

PLSR - Partial least squares regression analysis

PP- *Palmaria palmata* (Dulse)

PP10f/0.5s- Dulse sausage formulation containing 10% fat and 0.5% salt

PUFA- Polyunsaturated fatty acid

SAT - Sensory acceptance testing

SFA- Saturated fatty acid

SS- Sea spaghetti

SS10f/0.5s- Sea spaghetti sausage formulation containing 10% fat and 0.5% salt

TBARS- Thiobarbituric acid reactive substances

TPA - Texture profile analysis

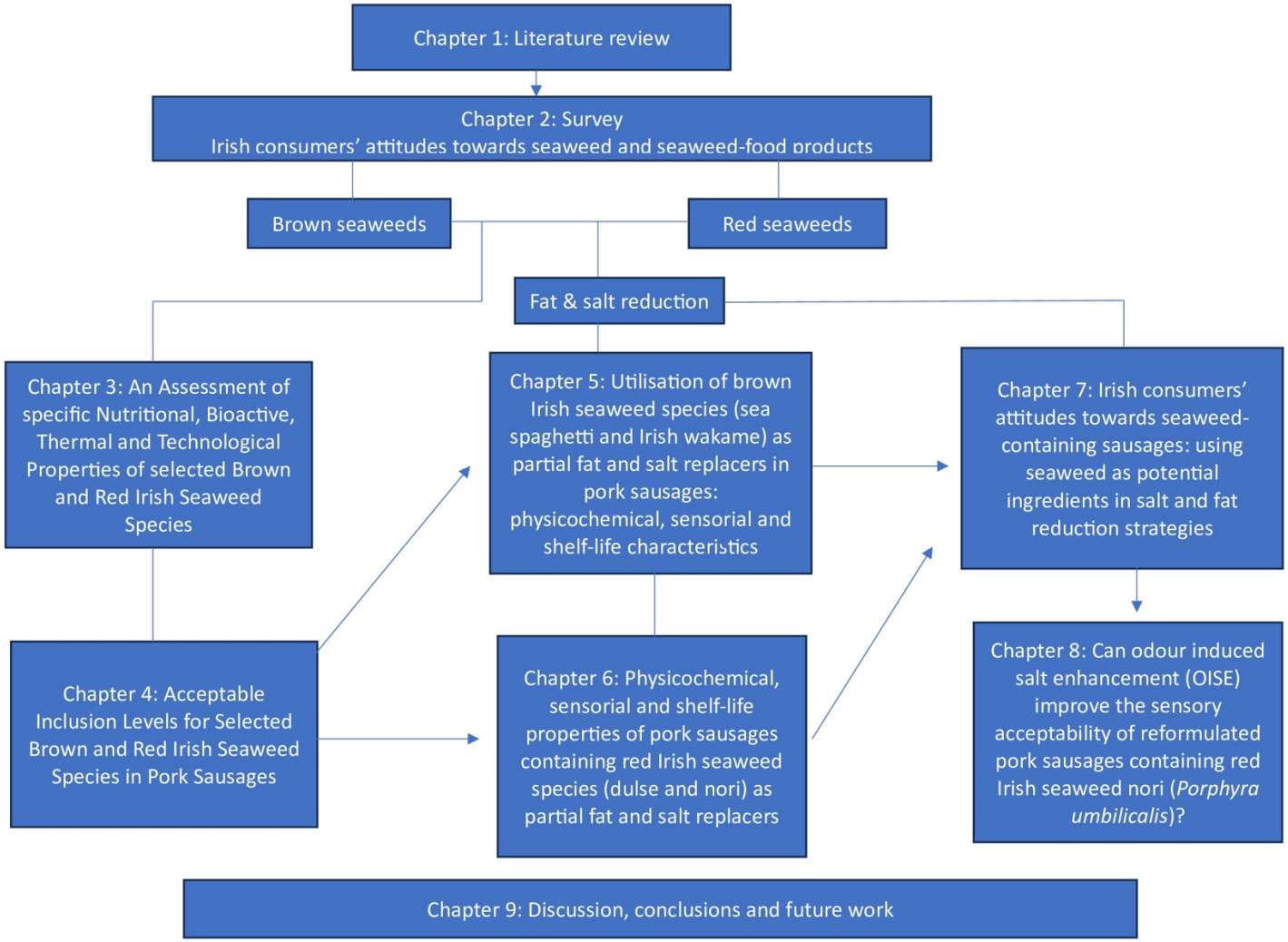
TPC- Total phenolic count

TVC- Total viable counts

VP- vacuum packaging

WHO - World Health Organisation

Schematic overview of thesis chapters



Research approach

The literature review in Chapter 1 provides a background for the experimental work carried out in Chapters 2 to 8. After conducting consumer research using an online survey presented in Chapter 2, the use of brown and red Irish seaweeds was implemented as fat and salt replacers in pork sausages in the subsequent chapters. In Chapter 3, the suitability of brown (sea spaghetti (SS) and Irish wakame (IW)) and red (dulse (PP)and nori) seaweed species harvested from the coast of Ireland as potential health-promoting techno-functional ingredients for use in food products was investigated. Seaweeds were characterised based on their nutritional, antioxidant, thermogravimetric and technological properties. These seaweed species were incorporated into pork sausages to determine their acceptable inclusion levels from a sensory perspective (Chapter 4). The maximum acceptable inclusion level for PP was 1% and 2.5% for SS, IW and nori. Edible brown (SS and IW) and red (PP and nori) seaweeds were incorporated at the maximum levels stated above into reduced fat (20%, 15% and 10%) and salt (1.5%, 1% and 0.5%) pork sausages (Chapters 5 and 6). The physicochemical and sensory characteristics of reformulated sausages were analysed. Based on sensory results, sausage formulations: SS10f/0.5s, IW15f/1.0s, PP10f/0.5s and nori10f/1.0s were selected. Subsequently, shelf-life evaluation (lipid oxidation and microbiological analysis) was performed on selected formulations stored under AP, MAP70/30, MAP80/20 and VP conditions. In Chapter 7, a consumer acceptability study on this optimised seaweed reduced-fat and -salt sausages SS10f/0.5s, IW15f/1.0s, PP10f/0.5s and nori10f/1.0s in comparison to experimental (EC) and commercial (CC) controls was conducted. In Chapter 8, an attempt to improve the acceptability of the least liked formulation (nori10f/1s) by consumers

was made using bacon stimuli (odour-induced saltiness enhancement (OISE) strategy) at 0.08%. Overall thesis conclusions and future work are presented in Chapter 9.

CHAPTER 1.

Literature review

1.1. INTRODUCTION

A new health consciousness is very evident among the food consuming public and consumers are now paying more attention to their diet and how it contributes to their overall health and longevity of life. The increase in the level of research carried out over the past two decades correlating diet and incidence of certain chronic diseases has shown that considered food selection can support and improve health. Consequently, it is not surprising that there is now considerable interest among consumers and the food industry in general in products that can promote health and well-being (Plaza *et al*., 2008), even if some of the reasons for doing so are market driven.

Meat and processed meat products are one of the significant sources of fat and salt in our diet, and *excessive* consumption increases the threat of attracting chronic diseases such as obesity, cancer, type 2 diabetes and cardiovascular diseases (IOTF, 2002; Jiménez-Colmenero *et al*., 2001; Weiss *et al*., 2010; WHO, 2003). The Department of Health and the Food Reformulation Taskforce (Ireland) aims to reduce the intake of saturated fat and salt in the Irish diet by 10% and listed sausages as one of the significant contributors of saturated fat and salt (sodium) in the Irish diet (FSAI, 2022). The meat industry now must face the double challenge of producing healthier processed foodstuffs with adequate technological and organoleptic attributes without negatively affecting the economic viability of the final product (Gullón *et al*., 2021).

Sodium intake of less than 2 grams (5g of salt) per day is recommended (WHO, 2012). An average of 2877 mg/day in men and 2134 mg/day in women has been reported as the mean dietary sodium intake in Ireland (Morrissey *et al*., 2020).

Salt and fat play significant roles in processed meats and reducing sodium and saturated fat content influences the final product in numerous ways. For instance, fat reduction in emulsion-type products was demonstrated to affect a variety of quality attributes, including texture, sensory and cook yield (Rogers, 2001). Aditionally, technological problems, including a reduction in particle binding, rubbery skin formation, production of a soft mushy interior and excessive purge have also been reported in products (Naga Mallika *et al*., 2009). Salt serves as a preservative and solubilises myofibrillar proteins during manufacturing which are responsible for increased hydration and water-binding capacity, increased binding properties of protein and improved texture (Offer and Knight, 1988). Several other researchers reported detrimental effects on general functions as described but also on flavour and inabilities in preventing microbial growth (Desmond, 2006; Totosaus and Pérez-Chabela, 2009). For all of these problems, meat product manufacturers have risen to the processing challenges and a number of companies have successfully commenced reformulating their recipes for commercial offering to consumers. They now offer lower levels of salt and fat or even higher levels of polyunsaturated fatty acids in processed meat products (Verbeke *et al*., 2010). As a consequence of this reformulation of products, many new, natural and alternative ingredients and products to salt and saturated fat are being investigated in an attempt to assist in producing healthier and better tasting processed food products. One promising category of such material is seaweed and seaweed-derived ingredients.

Seaweeds (marine macroalgae) have been consumed widely as food in East Asia (mainly in Japan, China and Korea) (MacArtain *et al*., 2007). They can be classified as functional foods due to their unique nutritional and chemical properties.

Epidemiological studies attribute regular consumption of seaweeds with several health benefits related to cardiovascular diseases, weight management, cancer, metabolic syndrome, Type 2 diabetes, digestive tract and bone health(Brownlee *et al*., 2012). Bioactive compounds present in seaweeds, include; polyphenols (phloroglucinol, phenolic acids and flavonoids), polysaccharides, pigments (carotenoids such as fucoxanthin and β-carotene), proteins (phycobiliproteins) or peptides, α-tocopherol (vitamin E), ascorbic acid (vitamin C) and a wide variety of minerals (Rodríguez-Bernaldo de Quirós *et al*., 2010; Farvin and Jacobsen, 2013; Agregán *et al*., 2017b). Bioactive properties associated with these compounds are related to antioxidant, antibacterial, antiviral and antifungal activities (Marinho-Soriano *et al*., 2006). Nutritionally, seaweed polysaccharides are a potential source of total dietary fibre (TDF). More specifically, the use of edible seaweed and its extracts as fat- and salt-replacers has been investigated. Fernández-Martín *et al*. (2009) investigated the use of *Himanthalia elongata* in pork sausages to replace animal fat and assessed the effects of incorporation on meat batter gelation. Additionally, López-López *et al*. (2010) added *Undaria pinnatifida* to beef patties to reduce salt and fat levels.

Salles (2006) demonstrated that taste could be enhanced via odour intensity, and conversely, the perception of taste could be enhanced by odour. This concept is termed "odour-induced saltiness enhancement" (OISE). The multisensory interaction between aroma and taste could be used to make up healthy low-salt food's final salty taste perception (Shen *et al*., 2022). Djordjevic *et al*. (2004) and Lawrence *et al*. (2009) reported an enhancement of perceived saltiness in products via the addition of ingredients such as; soy sauce, sardine, ham, chicken, peanuts and bacon odours.

Lawrence *et al*. (2009) also noted that several salt-associated odours could induce saltiness enhancement and showed that odours not associated with salt could in fact bring about a reduction in perceived saltiness, such as carrot odour.

1.2. MEAT COMPOSITION

Meat is a postmortem, edible skeletal muscle component derived from mammalian animal origin. The mammalian carcass is composed primarily of four parts: muscle, fat, tendons and ligaments, and bone. Commercially, muscle is the most important component and accounts for the largest weight. Generally, postmortem muscle becomes meat. Although fat, blood, and organs such as the heart, liver, pancreas, spleen, brain etc. are commonly processed, marketed, and used alongside muscle, they too are classified as 'meat products'. Meat quality is a complex trait which depends on the interactive effects of genotype, physiology, gender, environmental conditions, pre-slaughter handling and slaughtering procedure (Rosenvold and Andersen, 2003), collectively described as pre- and post-slaughter factors.

Postmortem muscles in meat vary in colour, ranging from a dark purplish-red to a pale, light grey. This variation is primarily the result of myoglobin concentration and other biological factors such as pH. Myoglobin is a protein physiologically crucial in transferring oxygen and carbon dioxide to and from muscles during their normal metabolic activities (Kauffman, 2012). Meat composition varies concerning its breed, type of feed being ingested, climatic conditions, and meat cut, which imparts a considerable difference in its nutritional and sensorial properties (Pereira and Vicente, 2013).

Generally, meat is a good source of various nutrients. It is high in high biological value protein and micronutrients like iron, selenium, zinc, and vitamin B12. Offal meats like liver are also high in vitamin A and folic acid (Biesalski, 2005). On average, most meat-derived muscles should contain approximately 1% ash (primarily represented by the elements potassium, phosphorus, sodium, chlorine, magnesium, calcium, and iron), 1% carbohydrate (primarily glycogen antemortem and lactic acid postmortem), 5% lipid, 21% nitrogenous compounds (predominantly proteins), and the rest (72%) as water (Kauffman, 2012).

Chemical compounds in meat muscle include free fatty acids, glycerol, triglycerides, phospholipids, and nonprotein nitrogenous components such as DNA, RNA, ammonia, amine groups, and vitamins. Fresh muscle comprises of water (free and bound), minerals such as K, P, Na, Cl, Mg, Fe, Zn, Cu and Ca, proteins, fat-soluble vitamins (A, D, E and K), water-soluble vitamins (thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, folic acid, and B12) and other organic entities.

Excluding water, the significant components in muscle are the contractile proteins, which make up the myofibrils (Kauffman, 2012). Muscle protein can be divided into four groups, the largest of which is myofibrillar. Myofibrillar proteins represent about 60% of the total proteins, whereas sarcoplasmic proteins represent 29%, stroma proteins 6%, and granular proteins 5%. There are nine known major myofibrillar proteins. The most important protein is myosin which represents 43% of the myofibrillar proteins, 26% of all muscle proteins, 23% of all nitrogenous compounds, and 5% of the fresh muscle mass. Lipid in the form of neutral triglycerides is stored in the adipose tissue cells, which accumulate around venules and arterioles in the interfascicular spaces. This fat, when visible within muscle, is called marbling.

Intramuscular fat, such as phospholipids and unsaponifiable constituents, e.g. cholesterol, are other forms of lipids found in meat (Kauffman, 2012).

Carbohydrates in meat are stored as "glycogen", predominantly in the liver and muscles but also in glands and organs to a lesser extent. Substantial quantities are present in the blood in the form of glucose. Glycogen has an indirect impact on meat colour, texture, tenderness and water-holding capacity. The conversion of stored glycogen to glucose and then from glucose to lactic acid is quite a complex process, and the action of hormones and enzymatic systems governs these modifications (Jensen *et al*., 2011). Despite meat containing only a small amount of carbohydrate, they are essential in developing flavour during cooking through caramelisation and Maillard-type reactions between reducing sugars and amino groups. Furthermore, carbohydrates are responsible for the brown colour of cooked meat (Belitz *et al.,* 2009).

1.3. PORK MEAT

Pork accounts for over 33% of global meat consumption (Lebret and Čandek-Potokar, 2022). Pork is one of the most widely eaten meats in the world. Pork is consumed in Europe (up to 75% in some countries) primarily in the form of a variety of processed products, either dried, cured, cooked (and consumed hot or cold), as reformed products (e.g. hams, bacon joints etc.) or restructured products (sausages, puddings, terrines etc.) (IFIP, 2021). The eating quality of pork is a combination of appearance, flavour, tenderness and juiciness (Aaslyng *et al*., 2003).

1.3.1. Nutritional composition of pork meat

The major nutrients present in pork meat are as outlined previously for meat in general. Pork has a high protein content and possesses all essential amino acids (AA), fats containing essential fatty acids, vitamins and minerals and other health-promoting compounds (e.g. antioxidants) (Lebret and Čandek-Potokar, 2022). Pork is rich in essential minerals and vitamins (Fe, Zn, Se, vitamins D, B1, B2, B6, and B12) with high bioavailability (Higgs and Pratt, 1998). While the protein content (18-22% of fresh muscle weight) and AA composition are stable and balanced for human consumption, the fat content and fatty acid composition vary significantly due to muscle-type and other edible parts of the carcass, as well as differences in carcass composition between animals of different breeds, gender and age (Lebret and Čandek-Potokar, 2022).

The three distinct layers of subcutaneous fat over the back of pigs vary in fatty acid composition (Kauffman, 2012). Constituent fatty acids in fat include saturated, monounsaturated, and polyunsaturated fatty acids (n-6 or ω-6 polyunsaturated fatty acid and n-3 or ω-3 polyunsaturated fatty acid). Intramuscular fat in pigs range between 1 and 10% of fresh muscle weight and is dependent upon muscle type. These lipids are comprised of triglycerides, phospholipids, and cholesterol (Schwob *et al*., 2020). The phospholipid content of oxidative (red) muscles is higher than that of glycolytic (white) muscles. Triglyceride content and fatty acid profile are highly variable. They are affected by muscle and husbandry factors such as genotype and sex, but primarily by pig diet, particularly in fatty acid composition (Lebret, 2008; Wood *et al*., 2008).

Saturated fatty acids account for 35-45% of total fatty acids, whereas monounsaturated fatty acids predominate in pig muscles and backfat, accounting for 42-52% of total fatty acids and nearly 60% in certain fatty breeds (Mourot, 2009; Poklukar *et al*., 2020). The primary tissue fatty acid (more than 40% of total fatty acid) is oleic acid C18:1. The proportion of polyunsaturated fatty acids is the lowest and most variable (5-20% of total) depending on carcass adiposity (breed, sex) and rearing (especially diet). The proportion of n-3 polyunsaturated fatty acid is generally much lower than that of n-6 polyunsaturated fatty acid (0.8-1.5% versus 5-18% of total fatty acid, respectively), resulting in an n-6:n-3 ratio of about 15, much higher than that recommended for human diets (<5), with the linoleic (C18:2 n-6) to linolenic (C18:3 n-3) acid ratio ranging between 15 and 30 for conventional pork (Mourot, 2009).

1.3.2. Pork muscle classification and uses

Common pork carcass cuts include the shoulder (roasting/braising), loin, side (belly for bacon manufacture) and leg (ham) muscles. Minced (ground pork) is generally a mixture of trimmed meat and fat, mainly from the shoulder and leg of the pig (Keenan, 2016). Breed, age, fatness, leanness and anatomical location influence the quality of pork cuts. Even within the same pig carcass, meat quality varies significantly from anatomical location to another(Chen *et al*., 2021). This variation results in different food and commercial values; for example, loin and leg (ham) is the pig carcass's most valuable cuts (Lebret and Čandek-Potokar, 2022).

The methods of cutting pigs for wholesale markets may differ slightly from region to region, but in general, four large sections are recognised:

1. Shoulder: accounts for approximately 25% of the carcass weight and is typically subdivided into two sections: upper ('Boston butt/blade Boston') and lower ('picnic'). The cuts are considered flavourful, but the connective tissue is significant due to the animal's considerable work in the shoulders. Therefore, roasting, braising, and marinating are popular cooking techniques with these cuts.
2. Loin: contains the rib, loin, and sirloin sections and accounts for 22% of the carcass weight. Typically, these are the most highly prized cuts as they are characterised by being very lean and tender (e.g., tenderloin). The loin can be whole or cut, that is, pork chops, while the ribs are noted to have higher meat content than their spare rib counterparts from the side.
3. Side (‘belly’): accounts for 23% of the carcass weight and contains both the spare rib and the belly. The meat is streaked with fat and often cured and smoked to produce bacon, such as pancetta. Spare ribs are usually trimmed of excess belly fat and slow-baked or barbecued.
4. Leg ('ham'): contains large muscles with low connective tissue content, accounting for 30% of carcass weight. Hams can be purchased either bone-in or boneless, and in some cases, the skins can be left on. Typically, hams are consumed cured or smoked, but fresh hams are also available (Keenan, 2016).

Pork in precut packages in various forms (chops, roasts, ground, single serving and cuts of loin, ham, neck, shoulder, ribs etc.) offer consumers fast and easy, ready-to-

cook products. These precut packages contribute to consumer satisfaction since consumers consider convenience the second most crucial pork attribute, after taste (flavour) (Resano *et al*., 2011).

1.4. PROCESSED MEAT PRODUCTS

Processed meat products were created to meet the demand for greater food supply and generating products with longer shelf-life than fresh meat. However, the generation of processed meats initially stemmed from the fact that quite a large proportion of meat originating on a carcass was inedible in that form and consequently, certain carcass parts and muscle-types were processed into added-value meat formats so that valuable resources were not wasted or lost. The formulation and manufacturing process affect processed meat products' shelf-life and physicochemical properties (Melro *et al*., 2020). Processed meat products include formats which can be described as reformed (ham, pastrami, corned beef etc. which do not physically break down the starting muscle structure) or restructured/comminuted (sausages, patties, burgers, nuggets etc. which physically break down the starting muscle structure) .

Ham is a high-quality and globally-popular processed meat product. Cooked ham, made entirely from pork, is brined before being pressed (or not) in moulds and then heated. The cooked ham's final quality is determined by both the raw materials and the process used, more precisely being; starting pork quality, the type of meat cut, visual leanness, type and amount of processing additives employed, the brine injection rate, the rate and extent of tumbling and the cooking and cooling processes

used (Delahunty *et al*., 1997; Tomović *et al*., 2013). According to consumers, the most desirable properties of high-quality cooked ham are its sensory characteristics (colour, aroma, and flavour), cohesiveness, firmness, and juiciness (Barbieri *et al*., 2016; Katsaras and Budras, 1993).

Bacon is another popular processed meat product. This product was initially developed as a method of preserving pork before the widespread use of refrigeration, and it is still widely used today. Bacon is made solely from pork belly cured with dry curing or wet-curing (brine) and then smoked or unsmoked. The quality of the pork bellies (soft bellies affect yield, sliceability and shelf-life of the final product) and the processing procedures influence the final quality of bacon (Larsen *et al*., 2009; Soladoye *et al*., 2015). Processed meat products involve a highly complex relationship between all ingredients employed, including; water, meat and processing aids mainly comprised of a series of different salts, including common table salt NaCl. Aside from these factors, raw meat pH and added non-meat ingredients influence the texture of cooked ham, as do the extent of heating (structural breakdown), the extent of proteolysis (degree of myofibrillar protein breakdown), the content of connective tissue present and the cooling treatment used (Barbieri *et al*., 2016; Desmond *et al*., 2002).











Figure 1.1 - Examples of processed pork products.

(Tesco Foods, Clonakilty Food Co., Henry Denny and Sons, Carroll's Cuisine UC)

1.4.1. Pork sausage composition and manufacture

Sausages represent one of the oldest forms of processed foods and their existence as a product format predates Roman times. The primary reason for the creation of the sausage format was as a means of processing large quantities of under-utilised muscle off-cuts, low visual lean meat cuts which were high in fat and connective tissue and excess fat deposits into a chopped and blended homogenous state and which was held together through the employment of tubular casings which originally would have been derived from membrane linings of the small and large intestines (and part of the processing by-product or offal).

Comminution of the fat is, therefore, a standard feature in sausage manufacture in an attempt to be sustainable in processing and minimising waste generation as well in the generation of energy-dense, flavoursome products. Sausages are defined in the United Kingdom as cylindrical products with hemispherical ends. The word "sausage" is derived from the Latin word salus, meaning salted, and sausages consisted of meat, blood, and other ingredients, which were filled into the animal gut or intestinal linings and preserved. The products' composition has remained relatively similar throughout the millennia. Meat is primarily responsible for binding the product together. It is modified by its treatment with salt and water and mechanical action to bind water, fat and other added ingredients into the final sausage matrix. The meat is chopped (restructured/comminuted) to varying degrees to help extract the myofibrillar proteins. Salt is added to extract the myofibrillar proteins, especially actin and myosin, because myofibrillar proteins are defined as being salt-soluble. The extracted proteins coat fat and water (and many other matrix components) forming a structure similar to an emulsion. At this point, other

ingredients and additives can be added to help improve technological performance or cost-effectiveness in sausage manufacture. Various cereals (flours, starches, bread, and breadcrumbs) have been traditionally used in sausages. More common today is the use of rusk, a product derived from biscuit dough that can be ground to various sizes, which is employed for texture and water-binding properties (up to three times its weight under ideal conditions). Other additives, such as polyphosphates, can be added to improve water binding and lessen cooking losses.

In contrast, other ingredients can be added to enhance flavour (monosodium glutamate and spices) and extend shelf-life (sulfur dioxide). Protein substitutes (blood plasma, skimmed milk powder, casein, whey etc.) can also be added for increased functionality in the absence of lower myofibrillar protein contents, subject to regulation. The resultant batter is filled into sausage casings and tied in sausage links. Modern sausage casings (skins) are usually made from one of the following:

1. Natural casings: cleaned intestines derived from the large and small intestines
2. Artificial casings: (a) regenerated collagen (derived from animal hide or other collagenous materials), (b) cellulose (plant wall material) or plastics (polyamine, polyethylene terephthalate etc), which is usually peeled off after cooking (skinless sausage-type).

Heating (if applied) stabilises the system by denaturing the proteins and gelatinising heat-labile proteins (collagen). There are broadly three recognised sausage categories (although strict definitions do not exist owing to product and regional diversity):

1. Fresh: Typically uncured and sold uncooked, they have a short shelf-life, and examples come with varying degrees of comminution (although they are most commonly coarsely comminuted). Common examples include British and Irish types of fresh sausage that contain varying levels of meat content.

2. Emulsion: These are usually made from finely comminuted meat/fat and are more highly flavoured than fresh sausages (spices). Emulsion sausages generally undergo additional processing, such as cooking, curing, and smoking/ drying. Therefore, they have an intermediate shelf-life. Popular examples include frankfurter, Vienna sausage (wiener), bratwurst, and saucisson.

3. Fermented/dried: Lactic acid is produced by fermentation of microorganisms introduced in the form of starter culture. These organisms were also historically significant in curing by reducing unrefined nitrate salts (saltpetre) to nitrite. Finally, the products usually undergo a drying step in the latter stages of manufacture. This step gives the products a long shelf-life (up to 1–2 years), but the process is complex and challenging to manage. Examples include salami, coppa, cervelat, and rosette (Keenan, 2016).

1.5. FUNCTIONAL MEAT PRODUCTS

Functional foods can be defined as foods that are used to prevent and treat specific disorders and illnesses, in addition to their inherent nutritional value (Özbay Özbaş and Ardıç, 2016). Functional meat products either contain dietary ingredients that improve health or fewer negatively health associated compounds (Beriain *et al*.,

2018; Diplock *et al*., 1999; Xu, 2001). These products are typically created by reformulating meat and incorporating health-promoting ingredients such as fibres, vegetable proteins, polyunsaturated fatty acids (PUFA), antioxidants, extracts, cereal by-products, fruits, legumes, spices, herbs, and lactic acid bacteria, either being used alone or in combination, for the enhancement of the functional value of meat and meat products (Kausar *et al*., 2019) or, omitting/reducing ingredient components such as salt, fat, cholesterol, phosphates, nitrates/nitrites etc.

For instance, because of their inherent functional and nutritional effects, meat products containing dietary fibres such as alginate, carrageenan, xanthan gum, pectin, cellulose, and chitosan are excellent meat substitutes (Biswas *et al*., 2011; Hur *et al*., 2009; Kumar *et al*., 2011) if selected and processed correctly. Besides improving meat products' nutritional benefits, dietary fibre enhances colour, texture, viscosity, WHC and sensory properties (Biswas *et al*., 2011; Campagnol *et al*., 2012). Dietary fibre can also be added to meat products to reduce the caloric value by fat replacement (Özbay Özbaş and Ardıç, 2016).

1.6. REDUCTION OF FAT IN PROCESSED MEAT PRODUCTS

1.6.1. Health impacts of saturated fat consumption

Constituent fatty acids in fat include saturated, monounsaturated, and polyunsaturated fatty acids (n-6 or ω-6 polyunsaturated fatty acid and n-3 or ω-3 polyunsaturated fatty acid). Animal fats are relatively high in saturated fatty acids. The fat content of pork sausages currently being sold in Ireland contains 24-28% fat.

Pork back fat, utilised in sausage formulations, contains saturated fat at up to 40% of total fat content (Asuming-Bediako *et al*., 2014).

Obesity, stroke, cardiovascular disease and cancer have been associated with the intake of excess saturated fatty acids and cholesterol (Kauffman, 2012). Hence, dietary recommendations target lower amounts of saturated fatty acid and more significant amounts of n-3 polyunsaturated fatty acid in foods such as meat products (especially linolenic acid C18:2) (Mourot, 2009). By 2025, the Department of Health and the Food Reformulation Taskforce has targeted reduction of saturated fat intake via the Irish diet by 10% (FSAI, 2022). The recommendations of many international organizations that advice on the reduction of total fat intake, and in particular, of saturated and trans fatty acids establish that total fat should not exceed 30% of total energy intake and saturated fats should represent less than 10% of the total caloric intake, with trans fats accounting for less than 1% (WHO, 2020).

1.6.2. Role of fat in processed meat products

Meat fat is a reservoir for flavour compounds and contributes to product texture (Juiciness and tenderness), thereby enhancing acceptability (Carrapiso, 2007; Egbert *et al*., 1991). Fats considerably affect meat processing in terms of binding, rheological, and structural properties (Choi *et al*., 2009). Reduced-fat content in finely ground meat products affects not only the chemical composition and nutritive value but also leads to a firmer, more rubbery, less juicy product with a darker appearance, reduction in particle binding, reduced cook yields, soft and mushy

interior, rubbery skin formation, excessive purge and shortened shelf life accompanied by higher production costs (Cavestany *et al*., 1994; Desmond *et al*., 1998; Jiménez Colmenero, 1996; Keeton, 1994; Kirchner *et al*., 2000; Paneras *et al*., 1996; Troutt *et al*., 1992).

The three-dimensional network created by solubilized myofibrillar proteins can be stabilized by animal fat, improving the softness and mouthfeel of processed meat products. A further benefit of animal fat is that it minimizes water loss during maturation or cooking, enhancing yield and juiciness. Animal fat is also critical for lowering production costs, which makes meat products more accessible to a larger percentage of the population (Câmara *et al*., 2020). Because it produces a range of volatile molecules that attach to receptors in the olfactory bulb (nasal cavity) and trigger a response, fat is also crucial for enhancing the flavour of meat products. Fat is the source of more than half of the volatiles found in beef products. The majority of volatile substances also have hydrophobic characteristics, making them more soluble in fat than in water. Unsaturated fatty acid oxidation is mostly caused by heat, which produces volatile chemicals that give meat products their distinctive flavour. Aldehydes, free fatty acids, ketones, alcohols, and hydrocarbons are the primary substances produced. They are essential for the flavour of meat products due to their low detection thresholds (Domínguez *et al*., 2022; Schädle *et al*., 2022).

Fat replacers can affect product flavour by contributing their own flavours, reducing the original aroma-generating substrate (fat) or altering the release of aroma compounds (Brewer, 2012). Low-fat meat products with fat replacers have acceptable processing parameters and sensory characteristics. However, flavour is

still an issue with their utilisation, and in practice, a reformulation of flavourings and spices is usually required (Jiménez Colmenero, 2000).

1.6.3. REDUCTION STRATEGIES FOR FAT IN PROCESSED MEAT PRODUCTS

Products manufactured predominantly from use of high visual lean meat, such as cooked ham and dry-cured bacon loin, generally have an animal fat content of less than 10% (Pateiro *et al*., 2019; Vargas-Ramella *et al*., 2020a). However, much higher levels of animal fat are found in most processed meat products, especially if they are restructured/comminuted meat products. Typically, meat products such as burgers, raw and dry-cured sausages, emulsified sausages, meat puddings, or pâtés contain between 20–35% animal fat (Franco *et al*., 2020; Vargas-Ramella *et al*., 2020b; Ozaki *et al*., 2021).

Fat replacers can also be classified by their composition: protein-based, lipid-based, or carbohydrate-based (Roth *et al*., 1999). Some of these replacers can also be classified as functional ingredients such as hydrocolloids, vegetable and connective tissue proteins, which can improve water binding and modify texture (Cierach *et al*., 2009). Strategies used to incorporate animal fat replacers can be performed by direct application or performing a pre-treatment (jellification or emulsification that may include other components such as oils and vegetable extracts) (Karwowska *et al*., 2021).

1.6.3.1. Short term strategy

Reduced-fat sausages contain approximately one-half of the traditional fat level (14-15g/100g product). Low-fat products must have no more than 10g fat/100g product, while extra-lean products must be under 5g fat/100g product (Pearson and Gillett, 1996). A short-term solution for providing reduced- or low-fat meat products is by formulating with leaner meats (trimmings from carcasses and cuts) and reduction of fat and caloric content by adding water or ingredients that introduce less or no calories (Mallika *et al*., 2009). However, using lean meat could change the sensory profile of products and potentially raise manufacturing costs and using water could result in similar negative sensory attributes as well as destabilising product shelf-life/safety through water activity elevation (Aslinah *et al*., 2018; Brewer, 2012).

1.6.3.2. Carbohydrate- based replacers

Gums, dietary fibre (inulin, fructooligosaccharides), maltodextrins, oatrim (hydrolysed oat flour), starches and healthy grains (chia and quinoa) are used as carbohydrate-based fat replacers in meat/poultry products (Domínguez *et al*., 2022; Tokusoglu and Ünal, 2003).

For instance, when dietary fibres are added to meat products, emulsion stability, water and fat holding capacity and cooking losses can all be improved. Dietary fibres are also quite effective at enhancing the texture and juiciness of meat products (Madane *et al*., 2019). Additionally, replacing some of the fat in meat products with dietary fibres is a great way to lower their energy content while maintaining satiety (Carvalho *et al*., 2019). Therefore, replacing animal fat in meat products with dietary fibres is a very effective way to lessen the technological and sensory flaws brought

on by fat reduction. Additionally, adding dietary fibres to meat products can help delay the onset of a number of chronic illnesses, including diabetes, obesity, and irritable bowel syndrome (Sofi *et al*., 2017).

Additionally, the ability of some gums to form gels at low concentrations, physically binding water into a three-dimensional structure, has been investigated. For example, carrageenans (to structure the added water), alginates, different starches (to structure the supplement of water), gums (xanthan, locust bean gum), micro-cellulose, pectins, konjac or short-chain poly fructose (inulin) have been employed (Wallingford and Labuza, 1983; Whiting, 1984; Foegeding and Ramsey, 1986). These compounds exhibit important properties, such as the ability to form a strong matrix and a high water-holding capacity, which leads to an increase in yield and an improvement in the texture of meat products (Kim *et al*., 2020).

1.6.3.3. Protein-based replacers

The animal fat content of meat products can be decreased by using animal proteins made from whey protein and collagen. These proteins are particularly successful at circumventing shortcomings brought on by fat reduction in meat products owing to positive technological qualities linked to substantial water and fat retention capacity and high emulsifying and gelling capacity (Domínguez *et al*., 2022). Whey protein can effectively replace animal fat in emulsified sausages, according to past and recent studies. Microparticulated whey protein (MWP) was employed by Ozturk-Kerimoglu *et al*. (2022) to replace 50% of the animal fat in emulsified sausages. The inclusion of MWP improved the technological properties of the emulsified sausages (improved emulsion stability and yield), lipid stability (reduced lipid oxidation), while

reducing total fat, calorie and increasing protein contents. Collagen has also demonstrated a strong promise for lowering the amount of animal fat in meat products. According to Sousa *et al*. (2017), the inclusion of collagen effectively reduced animal fat in emulsified meat products by 50% without lowering quality of the final product.

1.6.3.4. Lipid- based fat replacers

Asuming-Bediako *et al*. (2014) explored the use of vegetable, rapeseed and sunflower oils in an attempt to replace pork backfat in sausages. Replacing the pork backfat emulsion with rapeseed oil emulsion at a total fat content of approximately 12% decreased the total saturated fatty acid (SFA) composition in products from 38% to 14% (4.5 to 1.8g/100g product), increased the total monounsaturated fatty acid (MUFA) composition from 45% to 59%, and increased the total polyunsaturated fatty acid (PUFA) composition from 15% to 25%. Saturated fatty acids were reduced from 38% to 24% (7.2 to 4.8g/100g product) by substituting 20% of the pork backfat with rapeseed oil. With the exception of minor differences in product firmness and particle size, there were no significant differences in eating quality and overall preference.

Given that vegetable oils are primarily made up of mono- or poly-unsaturated fatty acids, they are highly susceptible to oxidative reactions. Microencapsulation is a popular method used to reduce lipid oxidation in food (de Almeida *et al*., 2018). The microencapsulation procedure effectively protects vegetable oils against environmental variables such as temperature, light and oxygen. The use of oil microcapsules as prospective fat replacers in various meat products has recently

been demonstrated to have significant promise. For example, fish oil microcapsules in frankfurter-type sausages (Domínguez *et al*., 2017) tiger nut, chia, and linseed oil microcapsules in deer pâté (Vargas-Ramella *et al*., 2020), chia and linseed oil microcapsules in burgers (Heck *et al*., 2017), and hazelnut oil in beef burgers (Alasalvar *et al*., 2022) were used as partial fat substitutes. According to these studies, oil microcapsules used as fat substitutes reduce fat content and positively modify product fatty acid composition without impacting sensory appeal.

1.6.3.5. Combination- based replacers

Edible mushrooms are one of many ingredients that possess a combination of nutrients and compounds that can be incorporated successfully into reduced fat meat products. Protein, fibre, and umami compounds are abundant in edible mushrooms (Pérez Montes *et al*., 2021). Therefore, these natural compounds have the potential to reduce the defects caused by a reduction in fat content in meat products. To prevent enzymatic browning, edible mushrooms must undergo a bleaching process prior to use in meat products (Kurt and Gençcelep, 2018). Patinho *et al*. (2021) conducted a study in which cooked mushroom (*Agaricus bisporus*) was used to replace 25, 50, and 75% of the fat content in beef burgers. The burgers made with *Agaricus bisporus* had a higher yield and a lower diameter reduction, indicating an enhancement in cooking properties, while the fat content was significantly reduced (from 15% to 4%). The replacement of 50 and 75% of fat with *Agaricus bisporus* enhanced the juiciness, tenderness, and flavour of burgers. However, it is important to note that beef colour was altered (increase in redness and decrease in

lightness), as was texture (softening) when mushroom was added to burger formulations.

Healthy oils (or oil mixture) immobilized in gels are produced to replace animal fat, such as hydrogels and oleogels. Oleogels are mainly composed of the oil selected to replace animal fat, which is gelled by adding small amounts of organogelators, normally at high temperatures (Martins *et al*., 2020). While hydrogels are formed without the use of high temperatures. This fact protects bioactive or thermal-sensitive compounds against oxidative degradations. Proteins, polysaccharides, hydrocolloids, and their combinations gel the emulsion hydrogel (Domínguez *et al*., 2021a, 2021b). Emulsion hydrogels and oleogels have highly diverse qualities depending on the type, characteristics, combination, and amounts of each gelling agent or oleogelator employed (Domínguez *et al*., 2022). In hydrogel emulsions, the oil content is ~50%, so the product's total fat content is expected to decrease significantly, while in oleogels, the oil content is usually greater than 90%, so the product's total fat content is not reduced (Domínguez *et al*., 2021b).

Recent trials were undertaken with beef burgers in which soybean oil oleogels replaced 50% of the animal fat. One study utilised ethyl cellulose and adipic acid as gelling agents (Adili *et al*., 2020), while another employed carnauba wax and adipic acid (Aliasl khiabani *et al*., 2020). In both cases, the reformulation (particularly in the adipic acid-containing oleogels) increased the yellow colour index, decreased the red colour index and enhanced the hardness of the beef burgers.

There appears to be a definite preference for alginate-based gelling agents among all available gelling agents that can be utilised in hydrogels (Domínguez *et al*., 2022). In this regard, it was demonstrated in a study that using an emulsion hydrogel of

alginate and carrageenan with olive oil for the partial or total replacement of animal fat in beef burgers did not impact lipid oxidation but did modify products redness and luminosity values and also decreased product hardness (Özer and Çelegen, 2020). In terms of nutritional quality, the reformulation increased moisture content, decreased fat content and energy value, while improving the fatty acid profile (decreases SFA while increasing MUFA and PUFA). Consumer acceptability scores for the modified beef burgers were significantly lower than those noted for control samples (Özer and Çelegen, 2020).

Another study carried out by Lyons *et al*. (1999) explored the use of mixed gels containing high gelling 35% (protein) whey protein concentrate (8%) and carrageenan (1.5%), with dry addition of tapioca starch (3%) produced final low-fat (<3% fat) pork sausages with similar characteristics to those of full-fat (20% fat) controls.

1.7. REDUCTION OF SALT IN PROCESSED MEAT PRODUCTS

1.7.1. Health impacts of salt consumption

Sodium chloride (NaCl) is the most common source of salt in the human diet, and processed foods and restaurant foods are estimated to account for 80% of the daily sodium intake in most of the Western world (Taormina, 2010). The average daily consumption of salt from foods by Irish adults has been estimated as 10g per day (providing 3.9g sodium) (FSAI, 2016).

Excessive sodium intake has been associated with hypertension, leading to cardiovascular diseases and stroke (Inguglia *et al*., 2017; Partearroyo *et al*., 2019). Studies have shown that consuming a large amount of salt can weaken the immune

system and cause a variety of disorders, such as autoimmune encephalomyelitis and lupus (Sharif *et al*., 2018; Wu *et al*., 2016). High salt intake can upset the gut microbiome, reducing the intestinal survival rate of Lactobacillus, and resulting in high blood pressure (Wilck *et al*., 2017). Additionally, high salt intake induces liver fibrosis by producing excessive reactive oxygen species (ROS) (Wang *et al*., 2016).

In Ireland, 20.5% of sodium intake is derived from cured and processed meats while the remaining 79.5% is derived from other sources which include; cereals and cereal products, soups and sauces, milk and cream and processed vegetables (including crisps and snacks) (FSAI, 2016). Fresh pork sausages retailed in Ireland typically contain 1.7-2.4% salt. To address the public health issues linked with salt consumption, the World Health Organization (WHO) have recommended that adults limit their salt (sodium chloride) intake to less than 5g/day (equivalent to 2g/day of sodium). The following salt levels for processed meats were set; 0.68% salt for uncured cooked meat products, 1.38% salt for sausages, 1.5% salt for frankfurters, 1.63% salt for ham and other cured meats, 2.88% salt for bacon and 0.75% salt for burgers (FSAI, 2017). Additionally, the Department of Health and the Food Reformulation Taskforce in Ireland aims to reduce the intake of salt in the Irish diet by 10% on or before 2025 (FSAI, 2022). These recommendations and the increased consumer awareness of the link between food and health have prompted the food industry to develop low-salt meat products that adhere to the proposed nutrition standards (Gullón *et al*., 2021).

1.7.2. Role of salt in processed meat products

Salt (sodium chloride- NaCl) is a versatile ingredient used in the meat processing industry due to its low cost and its various technological properties. Salt activates proteins to increase hydration and water-binding capacity; increases the binding properties of proteins, improves texture, increases viscosity of meat batters, facilitates incorporation of fat to form stable batters, enhances flavour and is bacteriostatic (Terrell, 1983).

1.7.2.1. Impact of salt on Sensory properties

Salt contributes to taste, and sensory characteristics, such as the aroma profile of foods, by increasing the volatility of aroma compounds or mouthfeel and affecting saliva's lubricating properties (Koliandris *et al*., 2010). Salt enhances the flavour of meat products, with Na+ being the main contributor of perceived saltiness and Cl- anion modulating perception (Miller *et al*., 1991; Ruusunen and Puolanne, 2005). The perception of salt involves activation of physiological processes such as salivation stimulation and gastric acid secretion (Mattes, 1997).

One of the limitations of low-salt meat products is that the perceived saltiness of the meat, in addition to the flavour intensity, is diminished (Ruusunen and Poulanne, 2004). While the loss of saltiness may be problematic for some products in general, specific issues arise for those that contain bitter-eliciting substances. In these cases, lower sodium levels lead to a diminished ability for bitterness suppression, which might result in a product that is overly bitter (Gaudette and Pietrasik, 2017). Additionally, saltiness boosts the perceived in-mouth scent of items, which, in the case of meat, can translate to an elevation of "meaty" flavour. This is an essential

component in the overall acceptance of meat products (Ruusunen and Puolanne, 2005). Seeing as salt has an effect on the physical properties of meat products, it also has an effect on the sensory and textural qualities of such commodities (Matthews and Strong, 2005).

1.7.2.2. Impact of salt on water holding capacity and texture of processed meats

The solubilization of the meat's functional myofibrillar proteins is one of the most important roles that salt plays in the production of processed meats (Desmond, 2006). As a result of this quality of muscle myofibrillar proteins, meat products can be produced to possess a wide variety of meat textures and is primarily the reason why we have so many different types of processed meat products available on the global market (Matthews and Strong, 2005). According to Cheftel, Cuq and Lorient (1989), the solubility of proteins in water is determined by the distribution of polar and nonpolar groups in the amino acid lateral chain, as well as the ionic species that are present in the solutions (Curtis and Lue, 2006). In low concentrations of salt, the myofibrillar (contractile) proteins of muscle, actin and myosin, are insoluble, but in high concentrations of salt, they are soluble (Schmidt *et al*., 1987). This activates the proteins, which increases their capacity to bind water and hydrate, ultimately leading to an improvement in texture through an increase in the binding properties of proteins (Terrell, 1983).

Increasing the water-holding capacity of the meat can reduce cook loss, thereby increasing the tenderness and juiciness of the meat product (Desmond, 2006; Tobin *et al*., 2012a). Increased solubilisation results in extracted proteins which, on cooking, form stable cross-links and bind pieces of meat together (Desmond, 2006). In finely

chopped or emulsified products such as sausages, frankfurters, bologna, etc., the salt-solubilised myofibrillar proteins, derived from the lean meat component, forms a sticky exudate on the surface of the highly comminuted meat pieces, which subsequently binds the meat pieces together through the formation of a gel-based continuous phase called the matrix and which also forms a protein film around fat globules in a form of encapsulation. This matrix of heat-coagulated protein traps free water and, consequently, retains the fat during the cooking process, which ultimately results in a product with quality attributes that are satisfactory (Desmond, 2006; The Salt Institute, 2013).

1.7.2.3. Preservation effects of salt

Aside from flavour improvement and meat functionality, salt is essential in regulating the growth of microorganisms, particularly in refrigerated ready-to-eat (RTE) foods. NaCl is one of the most efficient and diverse antimicrobial ingredients used in foods. Salt addition to foods is one of the most efficient methods for developing safe and nutritious food products (Taormina, 2010). Direct toxicity of Cl, elimination of oxygen from the medium, sensitisation of organisms to CO_2, and interference with the quick action of proteolytic enzymes are all factors implicated in the preservation qualities of salt (Rockwell and Ebertz, 1924).

The preservation of food through the use of salt dates back centuries before the invention of refrigeration (Matthews and Strong, 2005). The ability of salt to lower water activity (aW), which is the quantity of free water that is accessible for the growth of microbes, is what gives salt its antibacterial properties (Inguglia *et al*., 2017). The effect of salt on microorganisms is dependent on the quantity of salt

present in the aqueous phase of the food (Inguglia *et al*., 2017); hence, reducing salt levels improves water activity, which in turn increases the amount of water that is available for the growth of microorganisms. Salt's ability to preserve food also has the additional benefit of inhibiting the growth of pathogens like *Clostridium botulinum* and *Listeria monocytogenes* in products that have been vacuum packed and chilled (Matthews and Strong, 2005).

1.7.3. Reduction strategies of salt in meat products

Considering the significant technological role played by salt in meat processing, a global approach is necessary to reduce salt content in processed meat products (Albarracín *et al*., 2011). There are various approaches to reducing salt in processed meat products, which can be partial or complete.

1.7.3.1. The use of other ingredients

Studies have explored the reduction of salt in meat products such as corned beef by reformulating the product without using substitutes or replacers (Fellendorf *et al*., 2018). However, the use of replacers can significantly improve the quality and functionality of meat products. Additionally, use of replacement ingredients and their impact on product taste depends, not only on the type of replacer used, but also on the meat product-type and on its formulation (Fellendorf *et al*., 2016b).

Ingredients such as whole seaweeds, seaweed extracts and edible mushrooms have been explored as salt substitutes (Gullón *et al*., 2021; Myrdal Miller *et al*., 2014; Wong *et al*., 2017). Adding mushrooms to meat products usually results in an increase in moisture, cooking loss, colour change, loss of firmness, but with

acceptable sensory qualities (Pérez Montes *et al*., 2021). A study conducted by Vilar *et al*. (2020) revealed that the incorporation of seaweeds into frankfurters resulted in a decrease of lightness (L*), redness (a*) and yellowness (b*) values in comparison to the control sample. However, incorporation also reduced the sensory acceptance, hardness and chewiness of products.

1.7.3.2. The use of alternative salts

The use of mineral salts like potassium chloride (KCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), non-chloride salts such as phosphates, k-lactate, glycine, salts of organic acids such as sodium lactate, potassium lactate and sodium diacetate, and minor peptides such as L-ornithyl taurine or L-ornithyl-B-alanine and the methyl and ethyl esters of glycine have all been employed in reduced-salt processed meat products (Seki *et al*., 1990; Devlieghere *et al*., 2009; Weiss *et al*., 2010; Ripollés *et al*., 2011; Domínguez *et al*., 2017; Jin *et al*., 2020; Teixeira *et al*., 2021).

The partial substitution of NaCl with KCl generally has negative consequences on flavour and texture of the product. The main limitation for the use of KCl as a salt (NaCl) substitute is its metallic flavour (Inguglia *et al*., 2017). Additionally, concerns arise about the risks associated with a higher load of potassium, especially for those affected by conditions including type I diabetes, renal disease, and adrenal insufficiency (Khaw and Barrett-Connor, 1984).

According to findings, when used in fermented sausage, partial substitution of NaCl (over 40%) with mixtures of KCl/glycine or K-lactate/glycine resulted in flavour and texture deficiencies (Gelabert *et al*., 2003). The growth of pathogens in packaged meat products seems to be equally well-restrained by potassium, sodium, and

calcium lactates. For example, potassium lactate and sodium diacetate preserved the sensory quality and shelf-life of packed cooked meats while lowering the sodium chloride content by 40% (Devlieghere *et al*., 2009). In addition to having beneficial effects on water holding capacity, potassium lactate is antibacterial against *C. botulinum, Staphylococcus aureus* and *L. monocytogenes*, which may also lead to a better cook yield and improved texture for the cooked product (Stekelenburg, 2003).

1.7.3.3. Processing techniques

High-pressure processing (HPP), ultrasound, and pulsed electric field (PEF) are processing methods that have been utilized to reduce salt in meat products. Because they are less harmful to the environment and consume less energy than traditional food processing methods, they are described as "green technologies" (Chemat *et al*., 2017). Processing time (da Silva *et al*., 2020), shelf-life (Cichoski *et al*., 2015), protein structure (Bhat *et al*., 2018), additive diffusion (Bhat *et al*., 2020), sensory profile and nutritional quality of the food (Koubaa *et al*., 2016) can all be improved with employment of these technologies.

Processing techniques like high-pressure treatment have been noted to improve protein functionality; hence, meat products will require lower salt levels to attain maximum functionality (Rodrigues *et al*., 2015; O'Neill *et al*., 2018). Crehan *et al*. (2000) concluded that HPP is a viable technology that partially compensates for the reduction of salt levels in frankfurters. Some studies have also shown that HPP enhances the saltiness perception in meat products (Clariana *et al*., 2011; Ken *et al*., 2006) due to differential binding forces of NaCl within the product network and its

release in the mouth (Tamm *et al*., 2016), which in turn would permit salt reduction, as the saltiness perception would be increased.

Ultrasound technology has been applied for brining, marinating, tumbling, and cooking, with promising results as a useful strategy to reduce the salt content in meat products, such as dry-cured ham, bacon and chicken breast (Gómez-Salazar *et al*., 2021). Krasulya *et al*. (2019) proposed injecting raw meat with ultrasound in a low salt brine. The sonicated brine had a more even distribution of salt, expedited the physicochemical and biochemical processes during salting, which shortened the curing period and caused significant structural changes in the meat and improved the extraction of myofibrillar proteins.

A change in the structure of myofibrils, which can impact texture and water retention capacity, can be brought about by applying a pulsed electric field to meat products (Bekhit *et al*., 2016; Bhat *et al*., 2019). Bhat *et al*. (2020) were the first to evaluate the efficacy of a pulsed electric field for lowering sodium levels in processed meats. Pulsed electric field, according to the authors, had no effect on product colour, yield, oxidative profile, or microbiological stability. However, saltiness was enhanced by pulsed electric field treatment, which enhanced salt dispersion and sodium delivery, leading to enhanced feeling of saltiness during chewing.

1.7.3.4. Flavour enhancers

There are a number of flavour enhancing and masking agents commercially available as processing ingredients and the number and variety on the market continues to increase. These include; yeast extracts, lactates, monosodium glutamate (MSG) and nucleotides amongst others. Taste enhancers work by activating receptors in the

mouth and throat, which helps compensate for salt reduction (Brandsma, 2013). A commonly used salt enhancer is MSG, which contains high levels of glutamic acid and imparts a 'umami'-type taste to enhance the palatability and acceptability of savoury foods(Durack *et al*., 2008). Yeast autolysates are also commonly used in low-salt products, in particular, they mask the metallic flavour of KCl (Desmond, 2006).

Other strategies which have not yet been employed in meat products, but have potential for such purposes, include the use of flavour enhancers that do not have a salty taste but enhance the saltiness of products when used in combination with salt (OISE) (Lawrence *et al*., 2009), structural designs (particle size, morphology and spatial structure) of solid salts, and heterogeneous salt distribution which results in a significant enhancement of saltiness without the loss of acceptability (Sun *et al*., 2021).

1.7.4. Simultaneous reduction of fat and salt in meat products

The simultaneous reduction of fat and salt can influence multiple factors in processed meats. Hence, the use of ingredients that can compensate for the negative factors caused by the simultaneous reduction of fat and salt is encouraged. Tobin *et al*. (2012a, 2012b, 2013); Fellendorf *et al*. (2015, 2017) carried out studies on the reduction of fat and salt in meat products such as white pudding, black pudding, frankfurters, beef patties and pork sausages. These authors concluded that reduced-fat and -salt meat products were highly acceptable by consumers, regardless of alterations in physicochemical properties such as colour, texture and cooking loss.

Research into the use of replacers to improve the quality and sensory acceptability of reduced-fat and -salt meat products has also been carried out. In two separate

studies conducted by Fellendorf *et al*. (2016c, 2016a), ingredients such as wheat bran, sodium citrate, carrageen, pectin, potassium chloride (KCl), a combination of potassium chloride and glycine, carboxymethylcellulose, PuraQ® Arome NA4, seaweed wakame, a mixture of potassium citrate, potassium phosphate and potassium chloride, and waxy maize starch, were explored as fat- and salt-replacers in white and black puddings. These authors concluded that in white puddings, the formulations containing 10% fat and 0.6% sodium formulated with sodium citrate and a combination of potassium chloride and glycine were found to be acceptable by consumers. However, these samples were harder, had reduced fatness perception and higher spiciness perception. In black puddings, formulations with 5% fat and 0.6% sodium containing KCl, potassium chloride and glycine mixture, and seaweed, and formulations containing 10% fat and 0.4% sodium incorporated with carrageen were perceived as spicy and salty. Samples with 10% fat and 0.4% sodium containing potassium chloride and glycine mixture were also perceived as fatty. Samples with 5% fat and 0.6% sodium containing pectin and a combination of potassium citrate, potassium phosphate and potassium chloride, and samples containing 10% fat and 0.4% sodium with waxy maize starch were liked for flavour and overall acceptance.

1.8. SEAWEEDS (MACROALGAE)

Seaweeds are marine macroalgae, plant-like organisms in coastal areas attached to rock or other hard substrata. They are classified into Phaeophyta (brown algae), Rhodophyta (red algae), and Chlorophyta (green algae) based on their pigmentation and further classified based on the chemical nature of photosynthetic storage products, the organisation of photosynthetic membranes, and other morphological features (Kılınç *et al*., 2013). Macroalgae require different light intensities to perform photosynthesis, and the presence of these different pigments in algae is related to their sea habitat. Thus, green macroalgae, which can absorb large amounts of light energy, predominate in coastal waters, whereas red and brown macroalgae predominate at greater depths where sunlight penetration is limited (Bocanegra *et al*., 2009).

These multicellular vegetative organisms lack true roots and stem and range in size and morphology from about 70 m to 50 cm per day (Bocanegra *et al*., 2009; El Gamal, 2010). They are currently being promoted as the plant-based food of the future because they do not compete with food crops for cultivable land and freshwater resources, they are a good source of critical nutrients such as carbohydrates, protein, and minerals, and they are a rich source of health-promoting compounds capable of acting on a wide range of disorders and diseases (Bocanegra *et al*., 2009; Cardoso *et al*., 2014; Fitzgerald *et al*., 2011; Lordan *et al*., 2011). They are called "nutraceuticals" due to their nutrient potential, and antioxidant, antimutagenic, anticoagulant, anticancer, and antibacterial properties (Abirami and Kowsalya, 2011). Geographic location and local environmental conditions influence the biochemical composition of marine seaweeds (Rohani-Ghadikolaei *et al*., 2012).

1.8.1. Brown seaweeds (Phaeophyta)

The brown algae constitute large cartilaginous forms, which can cover large areas of the shore and finer forms, therefore having a sizeable morphological range. They range from giant kelp, about 20m long, to thick leather-like seaweeds from 2-4m long, to smaller species of 30-60cm long (McHugh, 2003). They may be olive green or shades of brown, from golden to dark brown (Wells, 2009).

Polysaccharides present in brown seaweeds are alginate (alginic and uronic acids), fucoidan (sulphated fucose), mannitol, laminarin (β-1, 3 glucan) and sargassan which are sources of dietary fibre and can improve digestive health (Holdt and Kraan, 2011; Saeed et al., 2021). Brown seaweeds are used as the raw material for extracting hydrocolloids and alginate. The more useful brown seaweeds grow in cold waters in the Northern and Southern hemispheres; however, they thrive best in waters up to about 20°C. They are found in warmer waters but are less suitable for alginate production and rarely used as food (McHugh, 2003).

1.8.1.1. Bioactive compounds present in brown seaweeds

Brown seaweeds contain a wide range of bioactive compounds and usually have the highest levels of phytochemicals (Gupta and Abu-Ghannam, 2011).Brown seaweeds are a source of antioxidant compounds, for example ascorbic acid, catechins, tocopherols, polysaccharides (laminarin and fucoidan), polyphenolic phlorotannins and carotenoids.

Phlorotannins are the major source of antioxidant activity in brown seaweeds and are usually present at 2- 10% in brown seaweeds. They are formed by the polymerization of phloroglucinol (1, 3, 5 – trihydroxybenzene) monomer units and

biosynthesized through the acetate–malonate pathway, also known as polyketide pathway (Li *et al*., 2011). Phlorotannins can be classified into four subclasses based on their monomer linkage; fuhalols and phlorethols (ether linkage), fucols (phenol linkage), fucophlorethols (ether and phenyl linkage), and eckols and carmalols (dibenzodioxin linkage) (Pádua *et al*., 2015). Phlorotannins also possess antibacterial properties against some pathogenic food-borne bacteria such as *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Staphylococcus aureus* and *Escherichia coli* O157:H7 (Braden *et al*., 2004; Eom *et al*., 2008; Nagayama *et al*., 2002). Phlorotannins help to protect algae from stress conditions and herbivores, and due to the health beneficial various biological activities of phlorotannins, marine brown algae are known to be a rich source of healthy food (Li *et al*., 2011).

Fucoxanthin is the primary carotenoid in brown seaweed and has been found to possess anti-inflammatory, anti-cancer and antioxidant properties (Pádua *et al*., 2015). This carotenoid is also one of the well-studied algal compounds in terms of anti-obesity effect (Gammone and D'Orazio, 2015; Miyashita *et al*., 2011). Other pigments present in brown seaweeds are violaxanthin, neoxanthin A and B, α-carotene, β- carotene, and chlorophyll a, C1 and C2 (Jeffrey, 1976; Peng *et al*., 2011; Rodríguez-Bernaldo de Quirós *et al*., 2010). The colour of brown algae changes to an attractive green colour upon heating. The colour transformation occurs immediately upon exposure to water at high temperatures, making the seaweed visually resemble commercially available green vegetables (Blikra *et al*., 2019).

1.8.1.2. *Himanthalia elongata* (Sea spaghetti)

H. elongata is called sea spaghetti, spaghetti der Mer, thong weed, buttonweed, sea haricots, and sea thong. Species are usually found in the eastern Atlantic from southern Norway to northern Portugal but are most common in Scotland, western Ireland and southwestern Britain. They initially appear during winter as shiny greenish-yellow buttons about 2cm in diameter attached to rock surfaces, generally with small yellow forked shoots of the new frond visible. The yellow-coloured fronds arise from the centre of these buttons and divide into long-paired strap-like branches. *H. elongata* lacks the strong 'sea taste' but has a nutty flavour (Morrissey *et al*., 2001).



Figure 1.2 *Himanthalia elongata*

(https://www.detoxtrading.co.uk/product/sea-spaghetti-seaweed-organic/)

1.8.1.3. *Alaria esculenta* (Irish wakame)

Alaria esculenta (Irish wakame), a large brown seaweed, is a member of the Alariaceae and belongs to the Laminariales (kelp), which populates sublittoral zones of Arctic and cold temperate coastal ecosystems (Lüning, 1990). Irish wakame has a heteromorphic diplohaplontic life history. An upright stipe and a long blade with a

midrib characterise the thallus of this brown seaweed. The morphology of lamina and stipe can vary widely between specimens due to wave exposure (Sundene, 1962). In Europe, efforts for large-scale cultivation of seaweeds have primarily focused on the kelp species like Irish wakame because of their ability to reach high biomass yields (Kraan *et al*., 2000).



Figure 1.3 *Alaria esculenta*
(https://myplantin.com/plant/7106)

1.8.2. Red seaweeds (Rhodophyta)

Red seaweeds are usually small, generally ranging from a few centimetres to about a metre in length. They have had a more diverse evolution than green and brown seaweeds. Many species cannot stand desiccation and dominate the inter-tidal rock pools. Others tolerate desiccation, such as the purple laver, which can often be seen stretched out like a dry black film over mussel beds on rocky beaches. Red seaweeds such as *Polysiphonia lanosa* are epiphytes, which grow on other plants for physical support. In this case, the epiphyte benefits from the host's buoyancy, lifting it closer to the sunlight.

Red seaweeds are not always red: they are sometimes purple, even brownish red, but botanists still classify them as Rhodophyceae because of other characteristics

(McHugh, 2003). Therefore, it is necessary to use fresh material to assist with the first line of taxonomic classification and to reduce confusion with some Phaeophyta species. The red colour of the seaweeds is due to the enormous amount of red phycobilin pigments (phycoerythrin and phycocyanin) overriding the green pigment chlorophyll (a). Other pigments in red seaweeds are pheophytin a, β- carotene and leutin (Munsu *et al*., 2021). A phycobilin can further be categorised as phycoerythrobilin (pink–red compound), phycocyanobilin (blue compound), phycourobilin (yellow compound), and phycoviolobilin (purple compound) (Kannaujiya *et al*., 2021).

1.8.2.1. Bioactive compounds present in red seaweed

The total polyphenol content (TPC) of red seaweeds is typically quite high. This level of total polyphenol content is higher than that which is found in many fruits and vegetables, but it is lower than that which is found in brown algae. There is, however, a significant amount of variation among the values, with a median of 127 (mg GAE/100g), a maximum of 5121 (mgGAE/100 g), and a minimum of 25.8 (mgGAE/100 g) (Gamero-Vega *et al*., 2020). Protocatechin, genetic acids, hydroxybenzoic acid, and bromophenols are the several forms of polyphenols that can be found in red seaweeds (Gamero-Vega *et al*., 2020). They also have diterpenes and sesquiterpenes phenolics (Edelmann *et al*., 2019). It has been observed that the amount of polyphenols present in red seaweed is proportional to the amount of antioxidant potential it possesses. Other antioxidants like ascorbic acid and carotenoids also contribute to the antioxidant capacity, although their contribution is significantly smaller than that of polyphenols (Edelmann *et al*., 2019; Nunes *et al*., 2017).

Red seaweeds contain large amounts of polysaccharides, especially within the cell wall matrix and these polysaccharides possess unique structural characteristics and biological properties, which make them attractive sources of food supplements, functional foods, and nutraceuticals. Red seaweed polysaccharides biological actions can be classified as antioxidant, immunomodulatory, anticancer, and prebiotic (Qiu *et al*., 2022). Polysaccharides present in red seaweeds include carrageenan, agar and cellulose (porphyran, xylans, floridean starch and sulphated galactan).

1.8.2.2. *Palmaria palmata* (Dulse)

This seaweed is known as Dulse, Dillisk, Shell dulse or seagrass. It is a common cold-water species found in the middle to lower shore in parts of Europe and the North Atlantic coasts of America. *P. palmata* has a reddish-brown to dull dark red colour. It appears tough and leathery in texture, with flat fronds of 10-50cm in length. The shape of the frond can vary considerably between an irregular fan shape and a single frond with lateral marginal leaflets. It is one of the most famous sea vegetables used in Ireland. *P. palmata* is harvested all year round, but usually between spring and autumn when the vitamin content is high. It is rich, salty, and has a nut-like taste and unique mouth feel (Morrissey *et al*., 2001).

Dried *P. palmata* contains more iron (6.4 mg) than 100 g of raw sirloin steak (1.6 mg) in an 8 g serving (Institut de Phytonutrition, 2004). Essential amino acids such as leucine, histidine, valine and isoleucine are present in *P. palmata,* and levels of isoleucine and threonine are similar to the levels in legumes (Fleurence, 1999). This red seaweed contains up to 45% of the pentose-related polysaccharide xylan. This polysaccharide contains xylose and related sugars, a dietary fibre that benefits food

applications (Institut de Phytonutrition, 2004). Besides being used as a sea-vegetable, *P. palmata* has been used in manufacturing antiperspirant products in the body care industry (Morrissey *et al*., 2001).



Figure 1.4 *Palmaria palmata*
(https://www.seaweed.ie/descriptions/Palmaria_palmata.php)

1.8.2.3. *Pophyra umbilicalis* (Nori)

Pophyra umbilicalis (nori) is also known as laver, purple laver, sloke, black butter, and purple sea- vegetable. They are red seaweeds usually found on all European coasts from the high to mid-intertidal, normally growing on rough-surfaced rocks, mussels and concrete breakwaters as well as on other seaweed species in wave-exposed places. They can tolerate very high wave exposure but are also found on more sheltered coasts growing on rocks buried in the sand. When dried, they resemble strips of black plastic sacks attached to rocks and usually grow up to 20-30 cm long. In their early stages, they appear green, brownish purple, and light brown chocolate before turning black, indicating their readiness for harvest (Morrissey *et al*., 2001).

Nori seaweed is relatively high in protein, which can be as high as 47% of the dry weight; however, these levels may vary according to season (Fleurence, 1999; MacArtain *et al*., 2007). They are usually processed into nori sheets and contain slightly more fibre than bananas in direct weight comparison (3.8 g versus 3.1 g per

100g, respectively). Nori also provides 9 mg of vitamin C in an 8g portion (Institut de Phytonutrition, 2004). The family of red *Porphyra* seaweeds contain the polysaccharides porphyran and floridean starch or floridoside at levels up to 48% and 42%, respectively, by dry weight, and these polysaccharides have been studied for food applications (MacArtain *et al*., 2007; Takahashi *et al*., 2000).



Figure 1.5 *Porphyra umbilicalis*
(https://www.seaweed.ie/descriptions/Poprhyra_umbilicalis.php)

1.8.3. Green seaweeds (Chlorophyta)

Green seaweeds are small, with a similar size range to red seaweeds (McHugh, 2003). They are found on both sandy and rocky beaches. Many can tolerate low salinity and will colonise areas where rivers meet the sea. The green colour of the seaweed is due to the green pigment chlorophyll required for light photosynthesis. Using only chlorophyll means green seaweeds need good light levels and will not thrive in shadowed areas. It does give them an advantage, the ability to live higher up shore without competition from the red or brown seaweeds. They often turn brown during the decomposition process and may appear brown in the field due to the external presence of epiphytic diatoms (Wells, 2009). Polysaccharides (dietary fibre) present in green seaweeds are ulvan and lignin.

1.9. USES OF SEAWEED

1.9.1. Food

Seaweeds are high in vitamins, proteins, carbohydrates, trace minerals, and other bioactive compounds (Kumar *et al*., 2008). Seaweeds have been consumed as food in East Asia (primarily in Japan, China, and Korea) since ancient times, and epidemiological studies have linked their regular consumption to a variety of health benefits (Brownlee *et al*., 2012). As citizens of these countries migrated to other countries around the world, the demand for seaweed for food migrated with them, increasing supply in America and Europe. A different type of seaweed is consumed in Ireland, Iceland, and Nova Scotia (Canada), and this market is growing. Seaweeds make up about 20% of the Asian diet and are eaten for the flavour rather than the nutritional value. However, seaweeds are used as food additives or extracts in the Western diet (Carvalho *et al*., 2009). In Southern Vietnam, seaweeds can be eaten raw, boiled, or dried, with sweetened green beans, jelly, crushed ice, and coconut milk (Tsutsui *et al*., 2005). Table 1.1 includes some edible seaweeds.

Table 1.1. List of some edible seaweeds

Subclassification	Genus	Common name
Brown algae (Phaeophyta)	*Alaria*	Kelp/ bladder locks
	Himanthalia/ Bifurcaria	Sea spaghetti, fucales
	Laminaria	Kelp/ kombu/ kumbu/ sea tangle
	Saccharina	Sugar wrack
	Undaria	Wakame

	Ascophyllum	Egg wrack
	Focus	Bladderwrack, rockweed
	Sargassum	Mojaban/Indian brown seaweed
	Hizikia	Hijiki
	Sargassum	Sea holly
	Eisenia	Arame
Red algae (Rhodophyta)	*Rhodymenia/ Palmaria*	Dulse
	Porphyra	Nori/ haidai/ kim/ gim
	Chondrus	Irish moss/ carrigeen
	Mastocarpus/ Gigartina	Stackhouse/ guiry
	Asparagopsis	Limu Kohu
Green algae (Chlorophyta)	*Ulvaria/ Enteromorpha*	Laver/sea lettuce/ sea grass/nori

1.9.2. Production of hydrocolloids

Another important application of seaweed is the extraction of hydrocolloid substances. Hydrocolloids are non-sticky crystalline substances extracted from the cellular wall that have large molecules and produce a viscous solution when dissolved in water. Agar, alginate, and carrageenan are three hydrocolloids made from red and brown seaweeds. These seaweed hydrocolloids are water-soluble carbohydrate that are used to increase the viscosity of aqueous solutions in order to form gels of varying firmness. They are also used to make water-soluble films and to keep products like ice cream stable. For example, the smooth texture of ice cream is achieved by using hydrocolloids, which prevent the formation of large ice crystals (McHugh, 2003).

Genera of red seaweed *Gelidium, Gracilaria, Pterocladis*, and *Grelidiella* are used in the production of agar, which is widely used as a microorganism growth medium, in biotechnological applications, in pharmaceuticals as an excipient in pills, and in the food industry as a gelling agent (Kılınç *et al*., 2013; McHugh, 2003). Carrageenans are extracted commercially from the carrageenophyte red seaweed genera *Kappaphycus, Gigartina, Eucheuma, Chondrus,* and *Hypnea*, with carrageenans accounting for up to 50% of the dry weight (McHugh, 1987). *Betaphycus gelatinae, Eucheuma denticulatum*, and *Kappaphycus alvarezii* are some other important carrageenan species (Lobban and Harrison, 1994). Carrageenans are commonly used in the food and baking industries as stabilizers, gelling agents, emulsifiers, and thickeners. They are also used as binders in toothpaste, thickeners and stabilizers in cosmetics, and smoothers in pet food (Renn, 1997). Matrixes are also used in drug delivery systems to control drug release, as well as microcapsules and microspheres (Li *et al*., 2014).

Alginates and alginic acid are extracted from brown seaweed, which distinguishes them from other seaweed hydrocolloids. Most brown seaweed species contain varying amounts of alginate. Species used for commercial extraction are *Laminaria* spp., *Macrocystis* spp., *Ascophyllum* spp., *Sargassum* spp. and *Fucales* spp., and alginate comprises up to 40% of the dry matter in these species (Draget *et al*., 2005; McHugh, 1987, 2003). In the food industry, alginates are used as stabilizers and thickeners (e.g., in jelly, drinks and desserts). They are also used as wound dressings and as matrices to encapsulate and/or release cells and medicine in the pharmaceutical and healthcare industries (Finotelli *et al*., 2010; Leslie *et al*., 2013; Paul and Sharma, 2004).

1.9.3. Animal feed

The unique bioactive compounds present in seaweed can be used to increase animal production and health. Macroalage as a supplementation of animal feeds has a long history and association with the domestication of various animal species (Evans and Critchley, 2014). In *Bellum Africanum*, written in 45BC, an unknown author records that "in times of scarcity, the Greeks collected seaweed from the shore and having washed it in fresh water, gave it to their cattle and thus prolonged their lives (Newton, 1951). Brown and red seaweeds were often fed to sheep, horses and cattle for 6 to 8 weeks of the year and up to 18 weeks in some cases, when forage was very scarce. There are also reports of seaweed being preserved as silage and used as winter feedstuff for sheep and cattle in the early 1900s (Black, 1955; Hallsson, 1964). The most common species used in animal feed applications is undoubtedly *Ascophyllum nodusum,* most likely because of its abundance, and proximity to processing infrastructure (Evans and Critchley, 2014).

Also, studies have evaluated the incorporation of seaweed species in aquafeeds, *Ascophyllum nodosum* (Nakagawa *et al*., 1997), *Porphyra* (Kalla *et al*., 2008; Soler-Vila *et al*., 2009), *Ulva* (Wassef *et al*., 2001), *Hizikia fusiformis* (Pham *et al*., 2006), *Gracilaria bursa-pastoris*, *Gracilaria cornea* and *Ulva rigida* (Valente *et al*., 2006) and *Padina arborescens*, *Sargassum siliquastrum* (Ma *et al*., 2005).

1.9.4. Fertilizer production

The use of seaweed as fertilizer has permitted the partial substitution of conventional synthetic fertilizer (Dhargalkar and Pereira, 2005; Hong *et al*., 2007; Khan *et al*., 2009; Zodape *et al*., 2010). Seaweed liquid extracts are applied as foliar spray, soil drench,

or in granular/ powder form as manure and soil conditioners (Blunden *et al*., 1996; Lingakumar *et al*., 2004, Thirumaran *et al*., 2009). Seaweed extracts have revealed the presence of a wide variety of plant-growth promoting substances such as auxins, cytokinins, and betaines (Khan *et al*., 2009), which influence the shoot and root system development (Durand *et al*., 2003; Stirk *et al*., 2004). Beneficial effects such as improved germination, deeper root development, increased frost resistance, increased nutrient uptake and changes in plant tissue composition, leaf quality, higher yields, general plant vigor, reduced incidence of insect attack, longer shelf- life of produce and improved animal health when livestock are fed treated crops or pasture and resistance to pathogens have also been reported (Khan *et al*., 2009; Abetz, 1980).

1.9.5. Biogas production

Research about energy has been focusing on finding new sources of energy and renewable energy as well as efficient utilization of energy in many processes (Sitompul *et al*., 2012). Seaweeds were reported to be biomass resources for biogas because they have good anaerobic biodegradability for biogas production due to the high content of carbohydrate (25-60%-w) (which are easily biodegradable sugars, such as alginate, mannitol and laminarin) and water (70-90%-w), low content of lignin (1-7%-w) and cellulose (Burton *et al*., 2009; Bruhn *et al*., 2011; Chang *et al*., 2010; Chynoweth *et al*., 1981; Horn *et al*., 2000; Kelly and Dworjanyn, 2008). Technical feasibility data on anaerobic digestion of algal biomass have been reported for a number of marine species such as Laminaria, Sargasum, Macrocystis, Gracilaria, Ulva and, more recently, microalgal strains. These studies have concluded that seaweeds

are suitable feedstock for anaerobic digestion processes and the production of renewable energy (Chynoweth *et al*., 2001; Kelly and Dworjanyn, 2008; Peu *et al*., 2011; Vivekanand *et al*., 2012; Zamalloa *et al*., 2012).

1.9.6. Cosmetics

Edible seaweed, *Alaria esculenta* (Verdy *et al*., 2011) and polysaccharides like laminaran, fucoidan and alginate derived from brown seaweed, such as *Fucus vesiculosus* and *Turbinaria conoides* (Je *et al*., 2009) have been suggested to prevent skin aging and cutaneous disorders (Wang *et al*., 2015). Also, red algae *Porphyra umblicalis* contains large amount of mycosporine- like amino acids (MMAs) that can absorb UV light, thus can be used as a sunscreen (Shick and Dunlap, 2002).

Bioactive compounds derived from marine algae have positive potential to be used as skin whitening agents (Thomas and Kim, 2013; Babitha and Kim, 2011; Kang *et al*., 2004). *Saccharina japonica, Chondrus cripus* and *Codium tomentosum* have been noted to absorb and retain moisture, have hydrating, therapeutic and moisturizing effects, and regulate water distribution in the skin, thereby protecting the skin from dryness respectively (Wang *et al*., 2013a, 2013b; Wang *et al*., 2015). Seaweeds can also act as preservatives to prevent the growth of fungi and bacteria, which may spoil cosmetics or harm users (Wang *et al*., 2015).

1.9.7. Water waste treatment

Brown macroalgae can be used in the treatment of zinc- containing wastewaters as natural cation exchangers (Pozdniakova *et al*., 2016). Also, *P. canaliculata, Laminaria hyperborea, A. nodosum and F. spiralis* were shown to be natural cation exchangers

for heavy metals removal from petrochemical wastewater (Cechinel *et al*., 2016). Macroalgae, *Stigeoclonium* was used in the treatment of horticultural wastewater (Liu *et al*., 2016).

The degradation of dyes present in wastewater by algae occurs through three different mechanisms, such as consumption of dyes for their growth, transformation of dyes to non- coloured intermediates or CO_2 and H_2O and chromophores adsorption on algae (Holkar *et al*., 2016). Species of seaweed which have successfully been used are brown algae *Stoechospermum marginatum,* green macroalga *Enteromorpha sp.* and green macroalgae *Cladophora* species (Kousha *et al*., 2012; Khataee *et al*., 2011, 2013).

1.10. HEALTH BENEFITS OF SEAWEEDS

There is low consumer awareness regarding the potential health benefits of seaweed (Kadam and Prabhasankar, 2010). Consuming either raw seaweed or extracts or seaweed in other food products is beneficial to health. The seasonal and environmental variances in the composition of the individual seaweeds must be put into consideration before making health claims (Bocanegra *et al*., 2009).

1.10.1. Cardiovascular diseases (CVDs)

Remarkably, the Japanese have the longest life expectancy in the world and the lowest rates of CVDs, and these scores have been partly associated with their different dietary patterns, which include the regular consumption of macroalgae (Shimazu *et al*., 2007, Yamori *et al*., 2001). A study investigated the effect of nutrition

on mortality in Japan associated seaweed consumption with protection against CVD (Iso and Kubota, 2007).

The consumption of seaweed and reduced risk of CVD has been tested largely within in vitro or animal models, with limited human data to corroborate the proposed anti-inflammatory, antiangiogenic, anticoagulant, and antiadhesive properties of seaweed components (Cumashi *et al*., 2007). Fucoxanthin, usually found in brown seaweed reduced blood pressure and stroke risk factors in rat models (Ikeda *et al*., 2003). Also, tropical seaweeds reduced the level of cholesterol, low-density lipoprotein, triglycerides, lipid peroxidation and erythrocyte glutathione peroxidase (Matanjun *et al*., 2010).

Research using in vitro and animal models suggest carrageenan from *Chondrus crispus* may inhibit platelet aggregation and have anticoagulant properties (Liu *et al*., 2012). Fucoidan and laminarin, which are isolated from *Laminaria* and *Ascophyllum spp.* have shown anticoagulant properties that may aid in the prevention of CVD and antioxidant, anti-inflammatory and anticoagulant properties, respectively (Miao *et al*., 1999; Østergaard *et al*., 2000; Berteau and Mulloy 2003; Heo *et al*., 2005). Brown *et al*. (2014) suggested that these findings may be helpful mediators in the prevention of heart disease.

Lipids from seaweed also have beneficial properties relevant to heart disease. Algal oil have protective and therapeutic properties against CVD because they are good sources of the long-chain omega-3 (n-3) polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Cohen *et al*., 1995; Manerba *et al*., 2010). These algal oils have the ability to act as potent antioxidants (Park *et al*., 2010) .

1.10.2. Cancer

It has been proposed that the consumption of dietary seaweed confers protection against cancer (Teas, 1981), which is based on the observations that cancer incidence is much lower in individuals who consume a seaweed- rich diet in comparison with those who consume a western- style diet (Ferlay *et al*., 2010).

Studies have shown the effect of seaweed consumption on breast cancer. Yang *et al*., (2010) reported the intake of gim, a Korean- style edible seaweed in the genus *porphyra,* consumed as a whole dried seaweed was associated with breast cancer risk in premenopausal women. The anticancer properties of gim may be due to its high iodine content (Yang *et al*., 2010). Seaweeds have been identified to contain levels of iodine between 4.3 and 2660 mg/kg (Lee *et al*., 1994). Iodine– rich seaweed has been used in the treatment of breast cancer in Traditional East Asian Medicine to soften tumours and reduce nodulation (Cann *et al*., 2000; Aceves *et al*., 2005). It was reported in a study done in Spain that low iodine intake was linked to an increased risk of breast cancer mortality (Majem *et al*., 1988). Seaweed may also exert protection against breast cancer development through its effects on estrogen metabolism (Teas *et al*., 2009). Seaweed consumption was associated with lower mortality from lung cancer in both men and women and pancreatic cancer in men (Iso and Kubota, 2007) and protective against stomach cancer (Hoshiyama and Sasaba, 1992).

1.10.3. Metabolic syndrome

Study done in animal models suggested a role for algae in the treatment and prevention of metabolic syndrome (Ichimura *et al*., 2013). Seaweed is one of the foods suggested that may protect metabolic syndrome, and was linked to a reduction in the increase of metabolic syndrome prevalence in women (Yeh *et al*., 2011). A study reported that the consumption of 4-6 g/day of *U. pinnatifida* (dried whole seaweed) reduced blood pressure and waist circumference (Teas *et al*., 2009).

1.10.4. Weight management

Obesity increases the risk of developing other diseases, including type 2 diabetes, hypertension, dyslipidaemia, and moderately increases the risk of developing osteoarthritis and coronary heart disease (Haslam, 2006).

It is known that seaweed is a good source of dietary fibre (MacArtain *et al*., 2007) and dietary fibre has been reported to aid weight loss by prolonging gastric emptying rate, hence, enhancing satiety and resulting in the reduction of food consumption (Kristensen and Jensen, 2011). Most of the dietary fibre present in seaweed is not taken up by the human body, therefore providing a low caloric value to diet (Rajapakse and Kim, 2011). Seaweeds also contain alginates, which with growing evidence suggest that it may attenuate appetite (Brown *et al*., 2014). Consumption of alginate- based drink reduced cholesterol and glucose uptake in obese and overweight individuals, resulting in lower blood cholesterol and glucose levels (Paxman *et al.,* 2008). Studies in animals have shown that fucoxanthin from brown seaweed reduced body weight and the percentage of abdominal white adipose tissue

(Maeda *et al*., 2008). Brown *et al*. (2014) suggested that other seaweed components other than alginate may have potential in altering fat mass and weight.

1.10.5. Type 2 diabetes

Type 2 or non- insulin-dependent diabetes is often due to prolonged obesity, alongside impaired glucose tolerance, hypertension, and hyperlipidaemia (Roberts, 2010). Diabetes studies using animals and *Undaria spp.* showed desirable outcomes, such as improvement in lipid status, reduction in inflammatory effects, attenuation of weight gain, and regulation of blood glucose (Murata *et al*., 1999; Maeda *et al*., 2009) . Seaweed consumption by Korean males showed decreased incidence of diabetes (Lee *et al*., 2010). A single ingestion of brown seaweed extract regulated insulin levels and sensitivity in healthy adults (Paradis *et al*., 2011). Consumption of alginate drink resulted in reduced blood glucose, serum insulin and concentration of plasma C-peptide in diabetic men, and agar consumption reduced body weight, body mass index (BMI) and total cholesterol levels in individuals with impaired glucose tolerance and diabetes (Torsdottir *et al*., 1991; Maeda *et al*., 2005). Also, the high consumption of fibre from dried whole seaweed supplements containing *Saccharina japonica* and *U. pinnatifida* (48 g/day) reduced fasting and post prandial blood glucose concentrations and altered lipid profiles (Kim *et al*., 2008). These studies conclude that these effects may help manage the symptoms of type 2 diabetes.

1.10.6. Digestive tract health

Certain seaweed- derived fibres can have positive effects on gut health (Vaugelade *et al*., 2000; Deville *et al*., 2004). Studies have also observed the potential probiotic

activity of low- molecular weight polysaccharides (Devillé *et al*., 2007; Ramnani *et al*., 2012). Marine- derived products, such as alginates, laminarin and agar, and structural components of macroalgae are evolving as novel sources of prebiotic carbohydrates (O'Sullivan *et al*., 2010). Alginate and carrageenan gum have been shown to increase probiotic survival, providing live bacteria with a physical barrier against adverse digestive conditions (Ding and Shah, 2009).

1.10.7. Bone health

Increase in the consumption of calcium- rich food sources promotes the maintenance of bone health and this would contribute to osteoporosis- which is a degenerative disease defined as reduced bone mass and microarchitectural deterioration and results in decreased bone strength and increased bone fracture risk (Tang *et al*., 2007; Bonjour *et al*., 2009; Harvey *et al*., 2010). Seaweeds such as *Ascophyllum nodusum, Laminaria digitata* and *Ulva spp* have higher calcium content than that of whole milk (MacArtain *et al*., 2007). Heated seaweed extract from *Cystophyllum fusiforme* and mineralized seaweed product extracted from red algae *Lithothamnion calcareum* exhibited beneficial effects in osteoporosis/osteopenia and osteoarthritic patients, respectively (Fujita *et al.,* 1996; Fujita, 1997; Fujita *et al*., 2000; Frestedt *et al*., 2008; Frestedt *et al*., 2009). Also, an active supplement from whole dried *Fucus vesiculosis, Laminaria japonica* and *Macrocystis pyrifera*, rich in fucoidans and polyphenols, showed positive results in a small- scale study (Myers *et al*., 2010).

1.10.8. Antiviral properties

There are links between low rates of infections and high rates of algae consumption (Teas *et al*., 2004). Sulphated polysaccharides of seaweed species exhibit antiviral properties. Fucoidan has been reported to inhibit the growth of a variety of viruses, while carrageenan has shown antiviral activity against herpes simplex virus type 1 and type 2, HIV-1 and human rhinovirus (Soeda *et al*., 1994; Aisa *et al*., 2005; Grassauer *et al*., 2008; Trinchero *et al*., 2009). A dietary study performed with extract from Tasmanian *U. pinnatifida*, ingestion was associated with reduction or disappearance of symptoms in those with active herpetic infection, and those with latent infection became free (Cooper *et al*., 2002).

With all the positive benefits of seaweeds, a large consumption can lead to negative effects. 8.5 g of seaweed a day would result in the exposure of individuals to about 0.2–6.7% of the toxic quantities of arsenic, mercury, lead and cadmium (Hwang *et al*., 2010). Iodine, which is found in high concentrations in seaweed can be infectious to an infant through breast feeding and has been documented as cases of neonatal iodine toxicity and consequent hypothyroidism (Crawford *et al*., 2010). Consumption of seaweeds have also been associated with increased risk of cancer, such as prostate cancer and thyroid cancer (Severson *et al*., 1989; Michikawa *et al*., 2012).

1.11. SEAWEED AS A FUNCTIONAL INGREDIENT

Using seaweed as an ingredient in meat products presents an opportunity for the industry to improve the products and address consumers' demands (Cofrades *et al*., 2010). Seaweeds contain various bioactive compounds with potential health benefits, thereby increasing their potential as functional ingredients in the formulation of healthier foods (Cofrades *et al*., 2013; Fleurence, 1999).

These macroalgae are an excellent source of various biocompounds such as polysaccharides, protein, omega-3 fatty acids, carotenoids, phenolic compounds, vitamins and minerals (Agregán *et al*., 2017b; Ciko *et al*., 2018). These phytonutrients are responsible for the several bio-activities and nutritional properties such as antiviral, antibacterial, antioxidant, anti-inflammatory, neuroprotective, antihypertensive, antihyperlipidemic, anticoagulant, prebiotic and anticancer properties (Rodrigues *et al*., 2015; Ryu *et al*., 2014; Wang *et al*., 2017). Accurately, the identification of this large number of active agents has encouraged the interest of researchers and the food industry to design seaweed-based functional foods that can help maintain human health, prevent diseases, and reduce the risk of chronic illness (Cofrades *et al*., 2017; Roohinejad *et al*., 2017).

Edible seaweeds offer exciting possibilities in the meat sector to develop functional foods (Moroney *et al*., 2013; Roohinejad *et al*., 2017). Table 1.2 shows a list of whole seaweeds, the forms they were used in, the level(s) added, and their function incorporated into processed meat products. Seaweeds have also been incorporated into other foods to improve biofunctional and nutritional qualities. A few examples are *Eucheuma* in fish cutlet (Senthil *et al*., 2009), *Sargassum marginatum* in pasta (Prabhasankar *et al*., 2009), *Himanthalia elongata*, *Porphyra umbilicalis*, *Saccharina*

latissima, *Ulva lactuca* and *Undaria pinnatifida* in milk (Nuñez and Picon, 2017), *Kappaphycus alvarezii* in muffins (Mamat *et al*., 2018), *Fucus vesiculosus* in bread (Arufe *et al*., 2018) and *Eucheuma denticulatum* in noodles (Koh *et al*., 2022).

Table 1.2. Meat systems formulated from whole macroalgae.

Meat system	Seaweed sample	Form	Level (%)	Function	Reference
Beef					
Patties	*Sargassum thunbergii* *Gelidium amansii*	Dried powder	1- 5	Added nutrients	(Chun *et al*., 1999)
Patties	*Undaria pinnatifida*	Dried powder	3	To improve reduced fat and salt patties	(López-López *et al*., 2010, 2011)
Patties	*Himanthalia elongata*	Rehydrated, blanched and ground	10, 20, 30, 40	Source of antioxidants and dietary fibre	(Cox and Abu-Ghannam, 2013)
Patties	*A. nodusum* *P. palmata* *Ulva sp.*	Dried powder	1,3 1,3 0.5	Source of antioxidants and antimicrobials	(Shortle, 2016)
Pork					
Batter/ emulsion	*H. elongata, U. pinnatifida* and *Porphyra umbilicalis*	Dried powder	2.5 5	To improve reduced salt batter/emulsion	(Cofrades *et al*., 2008)
Batter	*H. elongata*	Dried powder	3.4	To improve reduced fat batter	(Fernández-Martín *et al*., 2009)
Batter/ emulsion	*H. elongata, U. pinnatifida* and *P. umbilicalis*	Dried powder	5.6	To improve reduced salt batter/emulsion	(López-López *et al*., 2009)

Frankfurters	*H. elongata* and *Schizochytrium* sp.	Dried powder Oil	5	To improve reduced-fat frankfurters	(López-López *et al*., 2009)
Frankfurters	*H. elongata*	Dried powder	5.5	To improve reduced fat and salt frankfurters	(López-López *et al*., 2009)
Frankfurters	*H. elongata*	Dried powder	3.3	To improve reduced fat and salt frankfurters	(Jiménez-Colmenero *et al.,* 2010)
Breakfast sausages	*L. japonica*	Dried powder	1-4	Added nutrients	(Kim *et al*., 2010)
Patties	*U. pinnatifida, Ulva lactuca* and *Hizikia fusiformis*	Dried powder	1 2 4	Added nutrients	(Jeon and Choi, 2012)
Patties	*L. japonica*	Dried powder	1 3 5	To improve reduced fat patties	(Choi *et al*., 2012)
Frankfurters	*U. pinnatifida* *L. japonica* *H. fusiforme* *S. herbacea*	Dried powder	1	To improve reduced salt frankfurters	(Choi *et al*., 2015)
Black pudding	*U. pinnatifida*	Not stated	3.3	To improve reduced fat and salt black pudding	(Fellendorf *et al*., 2016b)
Breakfast sausages	*A. nodusum* *A. nodusum* *Schizochytrium sp.*	Dried powder	1 0.5 2	Source of antioxidants and antimicrobials	(Shortle, 2016)
Frankfurter	*H. elongata* *U. pinnatifida* *P. palmata* *P. umbilicalis*	Dried powder	1	To improve reduced salt frankfurter	(Vilar *et al*., 2020)

Chicken

Restructured steaks	*H. elongata*	Dried powder	3	To improve reduced salt steaks	(Cofrades *et al*., 2011)
Mechanically deboned chicken sausages	*Kappaphycus alvarezii*	Dried powder	2, 4, 6	Added nutrients	(Pindi *et al*., 2017)
Sausages	*Kappaphycus alvarezii*	Not stated	2.57 10	Added nutrients	(Babji *et al*., 2017)
Sausages	*Kappaphycus alvarezii*	Dried powder	0.5 0.75 1	Added nutrients	(Biswas *et al*., 2020)
Sausages	*Kappaphycus alvarezii* *Eucheuma denticulatum*	Not stated	0.25 0.5 0.75	Added nutrients.	(Biswas *et al*., 2019)
Sausages	*Kappaphycus alvarezi* *Sargassum polycystum*, *Caulerpa lentilifira*	Dried powder	2 4 6	Added nutrients	(Munsu *et al*., 2021)
Combination					
Patties (Chicken and Pork)	*Laminaria japonica*	Dried powder	2.5	To reduce fat and calories and to add functional components	(Oh and Lim, 2011)
Sausages (Beef and pork)	Brown macroalgae	Not stated	10 15	To improve reduced fat sausages	(Sălăgean *et al*., 2015)
Sausages (Turkey and beef)	AlgySalt® from *Lithothamnium calcareum*, *Laminaria*, *Enteromorpha*, *Ascophyllum*	Not stated	0.846 2	To improve reduced salt sausages	(Triki *et al*., 2017)

	nodosum, *Palmaria palmata*, *Fucus vesiculosus*, *Himantha lia elongata*, *Laminaria saccharina*, *Ulva lactuca*, *Chondrus crispus*, *Porphyra umbilicalis*, Palmaria/*Porphyra/Ul va* and *Undaria pinnatifida*				
Plant-based					
Sausages	*Gracilaria vermiculophylla*	Dried seaweed	0.8	Added nutrients	(Burri *et al*., 2011)

1.12. INFLUENCE OF SEAWEEDS ON PORK MEAT QUALITY AND SHELF-LIFE PARAMETERS

1.12.1. colour

Seaweeds can affect the colour of pork meat products by directly adding whole seaweeds/ extracts into formulation or by adding seaweeds/ extracts to the pig feeds. The levels of dietary incorporation of seaweed biomass are generally found to be below 4%, which are considered additive/supplement levels (Ribeiro *et al*., 2021). A previous study reported that feeding pigs with up to 4% *Macrocystis pyrifera* influenced meat colour, with the 4% group having less red intensity (a*) compared to either the control or the group fed with lower (2%) seaweed (Jerez-Timaure *et al*., 2021).

The presence of seaweeds conditions the colour of meat products, although to a different extent depending on the species, quantity added and meat product(Cofrades *et al*., 2008). Seaweeds contain fucoxanthin, β- carotene and phycobilin pigments (phycoerythrin and phycocyanin) that exert their colour directly onto the meat products when incorporated. For instance, the incorporation of 1% red (*Porphyra umbilicalis* and *Palmaria palmata*) and brown (*Himanthalia elongata* and *Undaria pinnatifida*) seaweeds into frankfurters resulted in reduced L*a*b* values, hence darker frankfurters (Vilar *et al*., 2020). Also, adding whole seaweed and sea tangle (*Lamina japonica*) reduced the L* and a* values; however, increasing the b* values in raw and cooked breakfast sausages (Kim *et al*., 2010). Colour changes can also be due to Maillard reactions, which are boosted in some pork products by adding seaweeds (Moroney *et al*., 2015).

1.12.2. Lipid oxidation

Several studies have reported the antioxidant and antimicrobial influence of crude extracts from seaweeds using simple and fast in vitro assays (Cox *et al*., 2010a; Rajauria *et al*., 2010). The potential of using seaweed powder and extracts against lipid oxidation in foods and oxidative stress in target tissues has been widely studied. Previous studies reported a high correlation between the total phenolic content and antioxidant activity (Chew *et al*., 2008; Rajauria *et al*., 2010; Wang *et al*., 2009). Seaweed phlorotannins have been suggested to scavenge free radicals, namely, superoxide, peroxyl and nitric radicals, and chelate ferrous ions (Chew *et al*., 2008; Kuda *et al*., 2005; Valentão *et al*., 2010; Wang *et al*., 2009).

Natural antioxidant extracts derived from marine sources such as seaweeds may be used as preservatives in the food and pharmaceutical industries (O'Sullivan *et al*., 2011). The anti-oxidative potential of laminarin, fucoidan, and laminarin/ fucoidan seaweed extract was evaluated in pork homogenates, horse heart oxymyoglobin, and the quality and shelf-life of fresh and cooked minced pork patties (Moroney *et al*., 2013, 2015). Seaweed extracts stated above reduced lipid oxidation in pork patties during cooking, owing to the formation of potential antioxidant melanoidins (Maillard reaction products) (Moroney *et al*., 2015).

1.12.3. Microbiological analysis

Seaweed produces metabolites with antimicrobial activities. These compounds show antiviral, antiprotozoal, antifungal, and antibacterial properties. The compounds extracted from green, brown and red seaweeds showing potent antimicrobial activity belong to polysaccharides (fucoidan and laminarin), fatty acids, phlorotannins,

pigments, lectins, alkaloids, terpenoids and halogenated compounds (Ford *et al*., 2020; Pérez *et al*., 2016). Seaweed and seaweed extracts containing these metabolites have been screened for their potential uses as alternative remedies for treating many infectious diseases (Abu-Ghannam and Rajauria, 2013). Previous studies have investigated seaweed extracts to be effective at inhibiting several microorganisms, including *E. coli* and *Salmonella* (Besednova *et al*., 2015; Pérez *et al*., 2016; Safhi, 2014; Singh *et al*., 2015).

The microbiological shelf-life of reformulated frankfurters with added whole seaweeds, *H. elongata*, *P. umbilicalis* and *P. palmata*, was similar to the control, which had 50% higher salt (Vilar *et al*., 2020). Hamzaoui *et al*. (2020) also reported a polysaccharide extract from green seaweed *Chaetomorpha linum* extended the microbiological shelf-life of beef sausages. They noted that its efficiency could be due to the biological activities of the green seaweed.

1.12.4. Sensory analysis

Mabeau *et al*. (1992) reported that the high levels of aspartic and glutamic acids in seaweeds are responsible for their distinct flavour (umami flavour) and taste. Mišurcová *et al*. (2014) also indicated that alanine, glycine and glutamic acid were the main components of seaweed flavour. The diversity of volatile compounds in seaweeds produces different odours, contributing to the 'seaweed flavour' attribute (Vilar *et al*., 2020). These volatiles has various odours that could be categorised as 'marine' or 'seafood' (Vilar *et al*., 2021). Other odours, such as 'fish', 'fatty', 'honey', 'green', 'floral', and 'spicy' have also been detected in various green, red, and brown seaweeds (Terezinha and Narain, 2018; Yamamoto *et al*., 2014). For instance, Vilar

et al. (2021) reported the presence of the compound 1-Octen-3-ol in brown and red seaweeds following gas chromatography olfactometry (GC-O) analysis. This compound has been shown to contribute to forming a seafood aroma (Van Durme *et al*., 2013).

Incorporating seaweed or its extracts in meat matrixes could present limitations associated with sensory characteristics of the products, such as appearance, taste, odour, texture and overall flavour. Sensory limitations (non-typical flavour and dry feeling) linked to the presence of seaweed (*H. elongata*) were observed in frankfurters (Jiménez-Colmenero *et al*., 2010; López-López, Cofrades, and Jiménez-Colmenero, 2009). Seaweed flavour was a factor that reduced the acceptability of the reformulated frankfurters as it was also classified as off-flavour (López-López, Cofrades *et al*., 2009; Jiménez-Colmenero *et al*., 2010; Vilar *et al*., 2020).

1.12.5. Texture

Seaweed polysaccharides are a potential source of soluble and insoluble dietary fibres. These compounds exhibit higher water-holding capacity than cellulosic (insoluble) fibres. Soluble dietary fibres can increase viscosity, form gels and act as emulsifiers (Elleuch *et al*., 2011). seaweed polysaccharides have been reported to have different biological activities and can improve the structure and strength of food products (Balboa *et al*., 2013). The main sulphated polysaccharides found in seaweeds include fucoidan and laminaran in brown seaweeds, carrageenan in red seaweeds, and ulvan in green seaweeds (Costa *et al*., 2010).

The brown seaweed, wakame, improved the water-binding properties and hence, made softer textures in both raw and cooked patties, effects which may be related to the main seaweed components, i.e. dietary fibre (41%) and minerals (36%) (Cofrades *et al*., 2008)

Kim *et al*. (2010) reported higher hardness values in sausages with increasing sea tangle included. Adding seaweeds to meat products such as patties has also been reported to increase hardness values (Jeon and Choi, 2012).

1.13. CONSUMER STUDIES AND CONSUMPTION TRENDS

1.13.1. Consumer attitudes to reduced- fat and salt muscle foods

Changes in the sensory properties of food caused by fat and salt reduction are difficult or impossible to avoid. The quality of reduced-fat and salt foods is usually somewhat different from their regular-fat counterparts; hence, consumers often relate fat reduction to a modified sensory profile and reduced pleasantness (Kähkönen and Tuorila, 1999).

Dietary salt and fat intake reductions are progressing by raising consumers' awareness. Education through public health campaigns (via television, radio, newspapers, brochures, internet, social media and press releases) focuses on health risks leading consumers to purchase healthier food products, encouraging cooking from raw ingredients and for them to use less salt and fat during cooking and at the table (He and MacGregor, 2007). Since the WHO first promoted the introduction of foods low in salt and fat, an increased number of food producers have taken the challenge to manufacture acceptable sensory salt and saturated fatty acids reduced foods (Fellendorf *et al*., 2018).

For instance, Fellendorf *et al*. (2018) surveyed consumer attitudes toward salt and fat-reduced food in Ireland and observed increased purchasing of salt-reduced food. However, fat-reduced food products were already better accepted by consumers. Generally, men, young adults (<30 years) and less educated people purchased less salt and fat-reduced foods and took less care in maintaining a balanced diet. No conflict was observed for traditional foods reduced in salt and fat content when their intrinsic sensory character is maintained. Most participants were aware of the health risks of a diet rich in salt and fat, though fewer participants transferred this

knowledge into their diet. A very high demand for more salt and fat-reduced foods on the market was observed, although the smaller range of salt and fat-reduced products and their higher prices might diminish consumer acceptance.

Another study in Ireland indicated that price and base meat products influence consumer purchase intention for processed meat products more than the reformulations applied. Salt and fat reduction positively influence purchase intention and health perception; surprisingly, this reformulation's negative impact on taste expectation is insignificant. Healthy ingredient enrichment can improve the health image of processed meat; however, the type of ingredient should be carefully selected to maximise the likelihood of consumer purchase. Processed meat consumers differ significantly in their openness to reformulations. Consumers who are positive (or negative) about salt and fat reduction are also positive (or negative) about healthy ingredient enrichment. The proportions of frequent eaters (at least once a week) were 76% for ham, 51% for pork sausages, and 26% for beef burgers (Shan *et al*., 2017).

In a study conducted by Guàrdia *et al*. (2006), consumers in Spain had a positive attitude towards low-salt meat products. Furthermore, higher costs for salt and fat-reduced foods can be generally expected as salt and fat reductions are achievable by stealth through increasing other ingredients (higher in quality), using replacers/enhancers and compensation for the lack of taste with flavour delivery agents (e.g. herbs, spices, lemon, onion/garlic, vinegar, etc.) and losses in yield (Fellendorf *et al*., 2018; Kilcast and Ridder, 2007; Talbot, 2011). It was also estimated that a 20% to 30% sodium reduction results in food cost increases of 5% to 30% depending on the type of food product (Dötsch *et al*., 2009).

1.13.2. Consumer attitudes to seaweed and seaweed food products

An investigation into the market for seaweed-based food products in the United Kingdom was carried out by Bouga and Combet (2015). They discovered 226 specific seaweed-based food products distributed across ten categories (bread, confectionery, condiments, drinks, noodles and pasta, salads, whole seaweed, snacks, soup, supplements, and sushi). Examples of seaweed products currently on the Irish market are seaweed noodles, pasta, crisps, biscuits and soup (Figure 1.2). Two attractive attributes of seaweed are its sustainability and healthy characteristics (Comparini *et al*., 2016).

Edible seaweeds are seen as a novel food product in European nations like Spain and France, yet seaweed consumption in Europe is still relatively low (Losada-Lopez *et al*., 2021). For instance, the consumption of edible seaweed in France is mostly through Asian-inspired products (soup miso, sushi and salad). This is mainly due to a need for more consumer knowledge regarding the possibility of using it in other foods (Lucas *et al*., 2019). The primary usage of seaweed globally is for human consumption (McHugh, 2003).

A study by Birch, Skallerud and Paul (2018) on Australian consumers noted that the Key drivers for seaweed consumption include health and nutritional benefits, taste, being natural, safe, and fresh. Also, critical barriers are a need for knowledge and familiarity and the perception that seaweed is expensive. In this study, females and younger, health-conscious consumers with higher household incomes and levels of education, who are more adventurous with food (neophilic), and who tend to snack and assign symbolic value to food are more likely to consume seaweed. Comparini *et*

al. (2016) also stated that less reluctance and more knowledge on the part of the consumers would contribute to increased consumption of seaweed products. Other surveys on seaweed consumption in many nations, including Australia, Sweden and India, have also been published (Birch *et al*., 2019; Gajaria and Mantri, 2021; Wendin and Undeland, 2020).



Figure 1.6 Examples of seaweed products currently on the market.
(Blue Evolution, Harvest Moon Foods, Lismore Food Company, Abakus Foods).

1.14. ODOUR-INDUCED TASTE ENHANCEMENT (OITE)

Odour-induced taste enhancement (OITE) is a phenomenon that derives from the integration of taste and odour into flavour perception. In this interaction, the taste intensity of a stimulus is increased simply by adding a tasteless retronasal odour (Linscott, 2015).

Several studies have demonstrated odour-induced taste enhancement (Djordjevic *et al*., 2004; Frank and Byram, 1988; Lawrence *et al*., 2009; Prescott *et al*., 2004; Sakai *et al*., 2001; Schifferstein and Verlegh, 1996; Wang *et al*., 2019). For instance, as early as 1988, Frank and Byram showed that a strawberry odour could increase the sweetness of whipped cream with sucrose. Recent studies on odour-induced sweetness enhancement have been conducted by Wang *et al*. (2019) using beef, vanilla and pomegranate aromas.

Caporale *et al*. (2004) noted that the bitter taste was enhanced in olive oil by cut grass odorant (*cis*-3-hexen-1-ol). Another study by Labbe *et al*. (2006) also reported that cocoa and vanilla flavourings induced bitterness in a cocoa beverage and caffeinated drink, respectively. This study concluded that taste–olfaction integration is product dependent and related to the food experience. Cheese aroma mixture containing ten volatiles significantly enhanced umami and bitterness. This study showed that the perceived interaction between taste and cheese aroma depended on taste type and concentration levels (Niimi *et al*., 2014). Other odour–taste interactions have been established, such as taste-induced odour enhancement (Lim *et al*., 2014; Linscott and Lim, 2016). Taste and odour pairs that were more congruent or likely to be experienced together, such as sweet and vanilla odour, were more likely to show odour enhancement by taste than incongruent pairs, like citric acid and

vanilla odour (Green *et al*., 2012; Lim and Johnson, 2012). Retronasal odour enhancement by taste was since replicated using various delivery methods, including sip and spit (Lim *et al*., 2014), odour jars (Fujimaru and Lim, 2013), and actual food systems (i.e. cherry beverage and vanilla custard) (Green *et al*., 2012) and by using various tastant concentrations (Fujimaru and Lim, 2013).

1.14.1. Neurological correlation between odour and taste

Several studies were performed to find neurophysiological correlates of odour–taste integration (Verhagen and Engelen, 2006). Endogenous mechanisms produce OITE in the brain. Several functional magnetic resonance imaging (fMRI) studies have investigated brain areas involved in flavour perception, leading to the identification of a relatively consensual flavour network (De Araujo *et al*., 2003; Rolls, 1997; Rolls and Baylis, 1994; Seo *et al*., 2013; Seubert *et al*., 2015; Small and Prescott, 2005). In these studies, supra-additive activations for the odorant-tastant mixture were found in high-level areas, in the orbitofrontal cortex, the dorsal mid-insula, and the perirhinal cortex (De Araujo *et al*., 2003; Seo *et al*., 2013; Seubert *et al*., 2015; Small *et al*., 2013). However, odour–taste convergence was also found in the primary gustatory cortex, more precisely in the anterior insula and frontal operculum (De Araujo *et al*., 2003; Seubert *et al*., 2015). Regarding these fMRI results, two views exist (i) one consists of a hierarchical integration, starting with a parallel unimodal encoding of odour and taste in their respective cortices and further elaborated by higher-order unimodal zones before converging onto multisensory integrative areas to form the flavour perception; (ii) while the second proposed that odour and taste are already integrated into primary olfactory and gustatory cortices (Prescott, 2012;

Small *et al*., 2013; Small and Prescott, 2005; Verhagen, 2007; Verhagen and Engelen, 2006).

1.14.2. Odour-induced saltiness enhancement (OISE)

Odour-induced saltiness enhancement (OISE) is one of many variations of OITE. OISE is a method used to reduce the salt content in food. It uses aromas/flavours to compensate for salt reduction in food. The multisensory interaction between aroma and taste could make up a healthy reduced-salt food's final salty taste perception (Shen *et al*., 2022). It has been demonstrated that taste can increase odour intensity, and conversely, the perception of taste can be enhanced by odour (Salles, 2006).

Food aromas such as soy sauce, sardine, beef, bacon, ham, goat cheese, chicken and tuna have been explored as OISE aromas in water solution (Batenburg and van der Velden, 2011; Chokumnoyporn *et al*., 2015; Djordjevic *et al*., 2004; Lawrence *et al*., 2009; Nasri *et al.,* 2011, 2013; Onuma *et al*., 2018; Sinding *et al*., 2021). Also, comté cheese, sardine and butter aromas have been used in a food model like cheese as odour-induced salt enhancers (Lawrence *et al*., 2011; Syarifuddin *et al*., 2016). The study by Syarifuddin *et al*. (2016) used sardine and butter aromas as congruent aroma-enhanced salt and fat in cheese.

1.15. CONCLUSIONS

This review highlighted the roles of fat and salt in meat products and the negative effects of fat and salt reduction. The consumption of fat and salt can lead to severe illnesses such as cardiovascular diseases, stroke, obesity and various cancers. It also examined the use of seaweeds and odour-induced saltiness enhancement (OISE) as strategies for reducing salt and fat in processed meat products, i.e. pork sausages.

Seaweeds may also improve processed meat products' functionality, nutritional value and shelf life as they contain beneficial nutrients, antimicrobials and antioxidants. However, the limitations of incorporating seaweeds into processed meat products include the colour, texture and flavour changes which can decrease consumer acceptability. Therefore, combining seaweeds and OISE can enhance consumer acceptability of reduced-fat and -salt-processed meat products by masking the overwhelming seaweed flavour. From the extensive literature review, no work has been carried out on the simultaneous replacement of salt and fat in pork sausages using Irish seaweed species reviewed and a combination of these strategies (seaweed and OISE); therefore, these two approaches are investigated in the current thesis.

1.16. THESIS OBJECTIVES

This thesis was carried out with the following objectives:

- Obtain consumer attitudes and knowledge data pertaining to seaweed and seaweed food products to determine the acceptability and consumption patterns **(Chapter 2).**
- Assess selected nutritional, bioactive, thermal, and technological properties of brown and red Irish seaweed species **(Chapter 3).**
- Determine acceptable inclusion levels for selected red and brown Irish seaweed species in pork sausages **(Chapter 4).**
- Utilise brown (sea spaghetti and Irish wakame) **(Chapter 5)** and red (dulse and nori) **(Chapter 6)** Irish seaweed species as fat and salt replacers in pork sausages: analysing the physicochemical, sensory and shelf-life characteristics.
- Evaluate consumer acceptability of optimised seaweed breakfast sausage products (from Chapters 5 and 6) compared to a commercially available product in the Irish market using a consumer sensory acceptance test **(Chapter 7).**
- Enhance the acceptability of the least acceptable seaweed formulation (from Chapter 7) by consumers using Odour-induced saltiness enhancement (OISE) **(Chapter 8).**

CHAPTER 2.

Irish consumers' attitudes towards seaweed and seaweed-food products

Abstract

The objectives of this study were to conduct a survey on Irish consumers' attitudes and knowledge towards seaweed and seaweed-food products. The consumer survey was developed using Microsoft Forms and circulated online through university mailing lists and social media websites. The survey consisted of 649 participants and findings showed that most participants (87.2%) trust new foods, 89.3% were not afraid to eat new foods. Participants in the age group 21-29 years old were more trusting (P=0.019) towards new foods and less intimidated to eating new foods (P=0.015). The majority (73.6%) of Irish consumers had consumed either seaweed or a seaweed-containing product, where 21-29 year olds had higher numbers of participants who had consumed either seaweed or seaweed-containing products compared to other age groups (P=0.009). The majority (97.5%) of older participants (50-59 year olds) had more knowledge about seaweeds having health and nutritious benefits than all other age groups (P=0.001). Irish consumers are familiar with seaweeds and seaweed-food products; hence, there is a potential for Irish consumers to accept other seaweed-food products such as seaweed sausages and can be further commercialized by the food industry.

2.1. Introduction

While consumers are moving toward healthier and more sustainable foods, many food companies are continuing to produce a significant number of new products to become more competitive, guarantee their viability, and create augmented added value (de Barcellos *et al*., 2009). This rapid development of new products may cause consumers to feel insecure and such products may be met with resistance and scepticism in the market (Grunert and Valli, 2001). Neophobia is a personality trait that influences consumers' acceptance of new products (Losada-Lopez *et al*., 2021). Currently, edible seaweeds and seaweed products are niche products that can be purchased on the market. Seaweeds are sold in various forms, such as raw, dried, milled or rehydrated. An investigation into the market for seaweed-based food products in the United Kingdom was carried out by Bouga and Combet (2015). They determined 226 distinct seaweed-based food products distributed across ten categories (bread, confectionery, condiments, drinks, noodles and pasta, salads, whole seaweed, snacks, soup, supplements, and sushi). Examples of seaweed products currently on the Irish market are seaweed noodles, pasta, crisps, biscuits, soup, condiments and supplements. Two interesting attributes of seaweed are its sustainability and healthy characteristics (Comparini *et al*., 2016). The socio-economic benefits of the sustainable cultivation of seaweeds have also been highlighted within the EU (Buschmann *et al*., 2017) and in Mediterranean countries, such as Morocco and Tunisia (FAO, 2018). Additionally, the fact that edible algae have nutritional and health properties that have evolved to accommodate our modern lifestyles explains why they are being referred to as "superfoods" (Losada-Lopez *et al*., 2021) . Consumption of seaweed is connected with several health benefits,

including; reducing blood pressure and preventing strokes. Additionally, seaweed is a significant source of protein, amino acids, minerals, and vitamins (Al-Thawadi, 2018; Prager, 2017) and allergens linked to seaweed consumption are rare when compared with fish (Fleurence *et al*., 2012).

Seaweeds are frequently consumed in Asia and are usually linked with Japanese and sushi dishes, but this is in stark contrast with consumption levels in North America and Europe. Edible algae are seen as a novel food product within Europe (Losada-Lopez *et al*., 2021). For instance, the consumption of edible seaweed in France is mostly through Asian-inspired products (soup miso, sushi and salad). This indicates a need for more consumer knowledge regarding the possibility of using it in other foods (Lucas *et al*., 2019). In actuality, the primary usage of seaweed globally is for human consumption (Cottier-Cook *et al*., 2016; McHugh, 2003). Less reluctance and more knowledge on the part of the consumers will contribute to increased consumption of seaweed products (Comparini *et al*., 2016).

Data from recent surveys on seaweed consumption in many nations, including; Australia, Sweden, France, and India, have been published (Birch et al., 2018, 2019; Gajaria and Mantri, 2021; Lucas et al., 2019; Wendin and Undeland, 2020). These studies show and elaborate the behaviour of consumers towards the consumption of seaweeds. Hence, it would be interesting to investigate Irish consumers' attitudes concerning edible seaweeds and seaweed products, as seaweeds are widely harvested in Ireland and have been used widely in coastal communities as alternative food sources, ingredients and natural medicines. Therefore, the objective of this study was to conduct a survey on Irish consumers' attitudes and knowledge towards seaweed and seaweed food products.

2.2. Materials and methods

2.2.1. Survey design and questions

In July 2021, a survey entitled 'Irish consumers' knowledge and attitudes towards seaweed and seaweed-containing food products.' was designed and developed using Microsoft Forms. The first page of the survey provided respondents with an information sheet and participants were asked if they were 18 years of age or above before proceeding to the next set of questions. Socio-demographic-based questions such as Irish residence, nationality, age, gender, approximate annual income and education level. Nationality selections were Irish or non-Irish, while age selections were 18-20, 21-29, 30-39, 40-49, 50-59 and ≥ 60. Approximate annual income ranged from ≤ €10,000 to >€50,000. The highest education level achieved selections were as follows: primary or secondary school (PSS), post-leaving certificate course/ level 6 FETAC/ HETAC course (PLC), third level course/ HETAC level 7/8 (TLC) and postgraduate course/ HETAC level 9/10 (PGC).

The next section of the survey contained consumption-based, knowledge and belief-based questions which focused on the eating habits of respondents. Tables 2.2 and 2.3 outlines these questions and their respective responses. Surveys were circulated online through university mailing lists and social media websites (Twitter, Instagram and LinkedIn). All subjects gave their informed consent for inclusion before participating in the survey. The survey was conducted following the Declaration of Helsinki, and the protocol was approved by University College Cork Social Research and Ethics Committee (Log 2021-107).

2.2.2. Statistical analysis

The completed questionnaire was exported into an excel spreadsheet where data was cleaned and imported to IBM SPSS statistics 28 for windows (SPSS, Chicago, IL, USA) software package. Demographic data were summarised in frequencies and percentages and presented in contingency tables. The knowledge level and attitude of the participants were determined by analysing data using descriptive analysis by age, gender, and educational level. Chi-square statistics were used to determine significance, and where statistical differences existed, the chi-square post-hoc test was further used to identify these significances. A 5% level of statistical significance was used for all analyses ($P<0.05$), which was adjusted to control type 1 error using Bonferroni correction. The adjusted P value = 0.05/number of analyses performed. Adjusted standardised residuals are tested against 1 degree of freedom (df) (MacDonald and Gardner, 2000).

2.3. Results and Discussion

Respondent demographics are shown in Table 2.1. A total of 651 individuals reviewed the survey, and 649 consented to participate and were 18 years old or above. Two responses were excluded due to incomplete data. Most respondents were between 21 and 29 (26.6%), followed by 40-49 (24.7%). The majority of respondents were female (61.4%), which was expected as women are more likely to participate in surveys (Curtin *et al.*, 2000, 2007; Fellendorf *et al.*, 2018; Porter and Whitcomb, 2005). Most participants had achieved a PLC level of education (37.7%) or a PGC degree (37.6%). 81.6% of participants were Irish, and 95.1% resided in Ireland. A majority (30.8%) of participants had an approximate annual income above €50,000.

Table 2.1. Respondent profile.

	Frequency (n)	Percent (%)
Age		
18-20	32	4.9
21-29	172	26.6
30-39	125	19.3
40-49	160	24.7
50-59	118	18.2
≥ 60	40	6.2
Gender		
Female	397	61.4
Male	231	35.7
Prefer not to say	19	2.9
Education		
Primary or secondary school (PSS)	77	11.9
Post leaving certificate course/ FETAC/ HETAC level 6 (PLC)	244	37.7
Third level course/ HETAC level 7/8 (TLC)	83	12.8
Postgraduate course/ HETAC level 9/10 (PGC)	243	37.6
Nationality		
Irish	528	81.6
Non-Irish	119	18.4
Do you reside in Ireland?		
Yes	615	95.1
No	32	4.9
Approximate annual income		
≤ €10,000	118	18.2
> €10,000 to 20,000	93	14.4
> €20,000 to 30,000	91	14.1
> €30,000 to 40,000	92	14.2
> €40,000 to 50,000	54	8.3
> €50,000	199	30.8
TOTAL	647	100

The survey findings showed that most participants (87.2%) trusted new foods and 89.3% were not intimidated in eating new foods (Table 2.2). Participants in the age group 21-29 years old were more trusting (P=0.019) towards new foods and were also less intimidated towards eating new foods (P=0.015). This may be due to this age group being young and more adventurous towards exploring new foods. Loewen and Pliner (2000) and Tuorila *et al*. (2001) also noted that younger people had lower levels of neophobia. Birch *et al*. (2019) also recorded younger participants as being more adventurous with food.

The majority (73.6%) of Irish consumers had consumed either seaweed or a seaweed product, where 21–29 year olds had a higher number of participants who had consumed either seaweed or seaweed products than all other age groups (P=0.009) (Table 2.3). As stated above, this age group seems open to new foods; hence they consume seaweed or a seaweed product. Birch *et al*. (2018) reported that 74% of Australian consumers had eaten seaweed. Palmieri and Forleo (2020) also reported that 57% of Italian respondents had eaten seaweed at least once in the past and reported that 76% were willing to eat seaweed in the future. They suggested that this may be due to a familiarity with some traditional Italian dishes that use seaweed or owing to the spread of Asian gastronomy in the country, and the fact that customers are eager to consume seaweed may indicate that consumers are becoming more responsive to novel foods, particularly those that provide healthier options or are more sustainable alternatives to traditional food sources.

Participants who had attained a postgraduate course/ HETAC level 9/10 (PGC) also had a higher number of participants who had consumed either seaweed or seaweed-containing products than other education degrees (P=0.013). Birch *et al*. (2018)

noted that Australian consumers with a university degree (i.e. undergraduates and postgraduates) are four-times more likely to eat seaweed products. The study also revealed that early adopters of seaweed food products in western societies are people with higher educational levels who are adventurous in their food choices and perceive seaweed consumption to have symbolic value. de Boer *et al*. (2013) also found that a higher level of education positively impacted the choice of snacks made from environmentally friendly proteins, including lentils and seaweed.

The majority (97.5%) of older participants (50-59 year olds) have more knowledge about seaweeds having health and nutritious benefits than the other age groups (P=0.001). Males (34.6%) and females (21.9%) were very likely, and 42.1% of females were somewhat likely (P=0.022) to consume seaweed or seaweed-containing products if they had not already consumed either. This shows that, regardless of gender, a percentage of Irish consumers who had not previously consumed seaweed or seaweed-containing products seem interested in eating seaweed.

The majority (32.5%) of consumers who took this survey indicated that consumption of seaweed or a seaweed product did so because of taste, flavour or “other” reasons. Participants (31.5%) who consumed seaweeds or a seaweed-containing products for "other" reasons (Table S2.1- See Appendix) did so because of the following reasons: medicinal, low caloric content, as part of cuisine or sushi or out of curiosity. Taste and flavour only accounted for 6.3%, and the combination of the following reasons: health benefits, source of iodine, source of omega 3 fatty acids, and plant-based (vegan/vegetarian) accounted for 11.9%. Consumer acceptance motivations can be related to traditional drivers (sensory, taste, cost, and convenience) or/and developing factors (health and wellness, safety, environment, animal welfare and

familiarity) (Boukid, 2021; de Boer *et al.*, 2013; Schösler *et al*., 2012; Siegrist and Hartmann, 2019).

A large number of consumers (22.6%) also stated that the seaweed product they had consumed was seaweed sushi rolls, and 9.6% had consumed raw seaweed (Table S2.2- See Appendix). Lucas *et al*. (2019) and Palmieri and Forleo (2020) stated that the consumption of edible seaweed in France and Italy was mostly through Asian-inspired products like sushi. Seaweed consumption in the form of sushi and as an ingredient or in snack foods such as seaweed-flavoured crackers has experienced commercial growth in recent times. The comparison of Norwegian and Japanese sushi consumers revealed that in addition to health benefits and convenience, eating sushi was considered as being "trendy" (Altintzoglou *et al*., 2016).

The top reasons why Irish consumers in this survey had not consumed seaweed or a seaweed-containing products were; (1) Never had the opportunity to (38.9%), (2) Lack of availability (25.8%), and (3) It is unpleasant (Taste, smell, texture and appearance) (10.7%) (Table S2.3- See Appendix).

Table 2.2. Label check and neophobia (attitude towards consumption) responses of consumers.

	Age (%)						P value*	Gender (%)			P value*	Education (%)				P value*	Total (%)
	18-20	21-29	30-39	39-49	50-59	≥ 60		M	F	Prefer not to say		PSS	PLC	TLC	PGC		
Q. Do you check food labels for ingredients?																	
Yes	81.25	79.7	81.6	85.6	82.2	85	0.804	78.4	84.4	89.5	0.115	78.3	85.7	82.7	82.4	0.670	82.4
No	18.75	20.3	18.4	14.4	17.8	15		21.6	15.6	10.5		21.7	14.3	17.3	17.6		17.6
Q. I only eat familiar foods																	
Yes	37.5	40.1	30.4	26.9	25.4	32.5	0.072	33.8	30.5	31.6	0.694	28.9	32.5	35.0	29.1	0.515	31.7
No	62.5	59.9	69.6	73.1	74.6	67.5		66.2	69.5	68.4		71.1	67.5	65.0	70.9		68.3
Q. I do not trust new foods																	
Yes	6.2	**20.3**$^{0.0007}$	11.2	10.0	8.5	15	**0.019**	13.0	12.6	15.8	0.917	4.8	18.2	14.0	12.7	0.071	12.8
No	93.8	**79.7**$^{0.0007}$	88.8	90.0	91.5	85		87.0	87.4	84.2		95.1	81.8	86.0	87.3		87.2
Q. I am afraid to eat new foods																	
Yes	3.1	**16.9**$^{0.002}$	11.2	6.2	7.6	15	**0.015**	8.2	11.8	15.8	0.281	3.6	10.4	12.3	11.5	0.156	10.7
No	96.9	**83.1**$^{0.002}$	88.8	93.8	92.4	85		91.8	88.2	84.2		96.4	89.6	87.7	88.5		89.3
Q. I do not explore foods from other countries																	
Yes	12.5	13.4	12	11.3	14.4	15	0.969	15.2	11.8	5.3	0.296	9.6	9.1	15.2	12.7	0.396	12.8
No	87.5	86.6	88	88.7	85.6	85		84.8	88.2	94.7		90.4	90.9	84.8	87.3		87.2

* P value; level of significance within a group. Superscript values indicate where the significance lies.

Table 2.3. Consumption, knowledge and belief of consumers on the intake of seaweed and seaweed products.

	Age (%)						P value*	Gender (%)			P value*	Education (%)				P value*	Total (%)
	18-20	21-29	30-39	39-49	50-59	≥ 60		M	F	Prefer not to say		PSS	PLC	TLC	PGC		
Q. Do you know seaweed can be consumed?																	
Yes	93.75	9.11	89.6	97.5	95.8	95.0	0.043	91.3	94.7	88.2	0.207	96.4	89.6	95.1	91.8	0.168	93.4
No	6.25	9.9	10.4	2.5	4.2	5.0		8.7	5.3	11.8		3.6	10.4	4.9	8.2		6.6
Q. Have you ever consumed seaweed or seaweed products?																	
Yes	62.5	**64.5**$^{0.002}$	74.4	77.5	82.2	77.5	**0.009**	70.6	75.3	70.6	0.428	67.5	64.9	71.6	**80.3**$^{0.003}$	**0.013**	73.6
No	37.5	**33.5**$^{0.002}$	25.6	22.5	17.8	22.5		29.4	24.7	29.4		32.5	35.1	28.4	**19.7**$^{0.003}$		26.4
Q. How do you feel about consuming seaweed?																	
Safe	84.4	77.3	79.2	76.9	80.5	72.5	0.526	77.6	79.7	73.7	0.246	88.0	74.0	75.7	78.7	0.693	78.2
Unsafe	0	0.6	0.8	0	0	0		0.3	0.4	0		0	1.3	0	0.4		0.3
Wary	12.5	17.4	14.4	16.9	11.0	17.5		14.9	16.5	10.5		8.4	19.5	17.3	14.3		15.3
Other	3.1	4.7	5.6	6.2	8.5	10		7.2	3.4	15.8		3.6	5.2	7.0	6.6		6.2
Q. Do you know seaweeds have health and nutritious benefits?																	
Yes	87.5	81.4	87.2	93.1	**97.5**$^{0.002}$	95	**<0.001**	86.6	91.7	78.9	0.042	91.6	87.0	89.3	89.8	0.823	89.5
No	12.5	18.6	12.8	6.9	**2.5**$^{0.002}$	5.0		13.4	8.3	21.1		8.4	13.0	10.7	10.2		10.5
Q. If you have ever consumed seaweed or seaweed products, what do you think about it?																	
Safe	78.1	86.0	89.6	88.1	83.9	82.5	0.199	87.0	85.9	84.2	0.970	88.0	84.4	87.7	84.8	0.649	86.2
Unsafe	0	0	0	0	0	0		0	0	0		0	0	0	0		0
Wary	6.3	4.7	4.8	1.9	6.8	7.5		3.9	4.8	10.5		1.2	6.5	3.7	6.2		4.6
Other	15.6	9.3	5.6	10	9.3	10		9.1	9.3	5.3		10.8	9.1	8.6	9.0		9.2
Q. If you have not consumed seaweed or seaweed products, how likely are you to consume either?																	
Very likely	9.4	23.8	28.0	34.4	22.9	30.0	0.397	**34.6**$^{0.0007}$	**21.9**$^{0.0005}$	31.5	**0.022**	21.7	27.3	25.5	29.5	0.393	26.7
Somewhat likely	53.1	37.8	40.0	31.9	39.8	32.5		31.6	**42.1**$^{0.003}$	15.8		41.0	33.8	36.6	38.5		15.9
Neither likely nor unlikely	6.3	10.5	8.8	14.4	13.6	10.0		12.1	10.8	15.8		9.6	9.1	11.5	12.7		11.5
Somewhat unlikely	21.9	16.9	15.2	13.8	16.9	15.0		15.2	16.1	21.1		14.5	16.9	18.9	13.1		37.6
Very unlikely	9.3	11.0	8.0	5.5	6.8	12.5		6.5	9.1	15.8		13.2	12.9	7.4	6.2		8.3

* P value; level of significance within a group. Superscript values indicate where the significance lies.

2.4. Conclusions

Consumers are primarily motivated to consume seaweed and seaweed products based on their familiarity, as they tend to consume only what they are knowledgeable in. A significant proportion of Irish consumers exhibited trust in novel food items, had no apprehension towards consuming unfamiliar foods, and had previously consumed either seaweed or a product containing seaweed. Nevertheless, individuals in the younger age group (21-29 years old) exhibited greater trust in unfamiliar foods and displayed less apprehension towards consuming novel foods. The survey results indicate that Irish consumers possess favourable attitudes and knowledge regarding seaweeds, seaweed products, and their associated health advantages. Consequently, there exists a promising opportunity for the utilisation, commercialization, and acceptance of seaweed-based products, such as breakfast sausages, within the Irish market.

CHAPTER 3.

An assessment of specific nutritional, bioactive, thermal and technological properties of selected brown and red Irish seaweed species

Abstract

Irish edible brown (*Himanthalia elongata* - sea spaghetti, *Alaria esculenta* - Irish wakame) and red seaweeds (*Palmaria palmata* – dulse*, Porphyra umbilicalis* - nori) were assessed for nutritional (proximate composition, salt, pH, amino acid, mineral and dietary fibre contents), bioactive (total phenolic content (TPC) and *in-vitro* antioxidant activity (DPPH and FRAP)), thermal (thermogravimetric analysis (TGA)) and technological (water holding (WHC), oil holding (OHC) and swelling capacities (SC)) properties. Red seaweeds had higher ($P<0.05$) protein levels, while brown seaweeds possessed higher ($P<0.05$) moisture, ash, insoluble and total dietary fibre contents. Nori had the lowest ($P<0.05$) salt level. Seaweed fat levels ranged from 1-2% DW. Aspartic and glutamic acids were the most abundant amino acids, the total amino acid (TAA) content ranged from 4.44- 31.80%. Seaweeds contained numerous macro (e.g. Na) and trace minerals. The TPC, DPPH and FRAP activities followed the order: sea spaghetti $\geq$ nori > Irish wakame > dulse ($P<0.05$). TGA indicated maximum weight loss at 250 ^{o}C. Dulse had the lowest ($P<0.05$) WHC and SC properties while dulse and nori had higher ($P<0.05$) OHC than the brown seaweeds. Results demonstrate potential of seaweeds as functional food product ingredients.

3.1. Introduction

Seaweeds (marine macroalgae) are divided into three significant phyla or by colour; Phaeophyta (brown algae), Rhodophyta (red algae) and Chlorophyta (green algae). Since ancient times, macroalgae have been consumed widely as food in East Asia (mainly in Japan, China and Korea) (MacArtain *et al*., 2007). Seaweeds can be classified as functional foods due to their unique nutritional and chemical properties. Epidemiological studies attribute regular consumption of seaweeds with several health benefits related to cardiovascular diseases, weight management, cancer, metabolic syndrome, Type 2 diabetes, digestive tract and bone health (Brownlee *et al*., 2012).

Bioactive compounds present in seaweeds include polyphenols (phloroglucinol, phenolic acids and flavonoids), polysaccharides, pigments (carotenoids such as fucoxanthin and β-carotene), proteins (phycobiliproteins) or peptides, α-tocopherol (vitamin E) and ascorbic acid (vitamin C) (Agregán *et al*., 2017b; Farvin and Jacobsen, 2013; Rodríguez-Bernaldo de Quirós *et al*., 2010). Bioactive properties associated with these compounds are related to antioxidant, antibacterial, antiviral and antifungal activities (Marinho-Soriano *et al*., 2006). Nutritionally seaweed polysaccharides are a potential source of total dietary fibre (TDF). The TDF content of seaweeds is classified into soluble and insoluble fibre fractions. TDF is made up of a range of structural and storage polysaccharides, and intercellular mucilage.

Several edible brown (e.g. *Ascophyllum nodusum*- Egg Wrack, *Himanthalia elongata*- sea spaghetti and *Alaria esculenta*- Irish wakame) and red (e.g. *Palmaria palmata*- dulse, *Porphyra umbilicalis*- nori and *Chondrus crispus* - Irish moss) seaweed species are currently available on the Irish market in dried (whole or milled) form. Seaweeds

consumed as foods are often referred to as 'sea vegetables'. Sea spaghetti has a very mild taste and flavour when soaked and consumed as a salad ingredient. Irish wakame (*Alaria esculenta*), also known as 'dabberlocks' is commonly served either as a vegetable or salad leaf. The colour of brown seaweed is mainly due to the presence of fucoxanthin, an orange carotenoid, which has been reported to possess antimicrobial and antioxidant properties (Rajauria and Abu-Ghannam, 2013). Polysaccharides in brown seaweeds (alginate, laminaran, sulphated fucans (fucoidan), cellulose and mannitol) represent up to 84% of the total dry weight (Schiener *et al*., 2015). The soluble dietary fibre (SDF) fraction in brown seaweeds is comprised of laminarin, fucoidan and alginate, while the insoluble dietary fibre (IDF) is made of cellulose. Other components of the IDF fraction in brown seaweeds are uronic acids from alginate and neutral sugars (Lahaye and Kaeffer, 1997; Rupérez and Saura-Calixto, 2001).

The red seaweed dulse (*Palmaria palmata*) is one of the most widely used and consumed seaweed species in Ireland and is generally eaten dried and uncooked. Nori (*Porphyra umbilicalis*) is usually deep red to purple in colour with a lettuce-like appearance. Red phycobilin pigments (Phycoerythrin and phycocyanin) mask the green pigment chlorophyll a and beta-carotene and are responsible for the colour of red seaweed species (Gupta and Abu-Ghannam, 2011). Polysaccharides (SDF) present in red seaweeds are sulphated galactans (agar and carrageenans), representing approximately 70% of the cell wall constituents. The remaining polysaccharides (IDF) are composed of cellulose (7-24%) (Barsanti and Gualtieri, 2014). However, the IDF fraction of nori contains insoluble mannan and xylan.

From a technological perspective, brown and red seaweed types have been incorporated into foods to improve nutritional quality and food product functionality. For example; the influence of *Fucus vesiculosus* on the physical and textural properties of wheat bread (Arufe *et al*., 2018), the fortification of *Eucheuma cottonii* on the nutritional properties, iodine content, and glycemic index of pasta (Firdaus *et al*., 2017), the effect of *Kappaphycus alvarezii* on the physico-chemical properties of muffins (Mamat *et al*., 2018), the impact of *Himanthalia elongata* (sea spaghetti), *Undaria pinnatifida* (wakame), *Palmaria palmata* (dulse) and *Porphyra umbilicalis* (nori) on reduced salt frankfurters (Vilar *et al*., 2020).

A deeper knowledge of the unique compositional and nutritional properties associated with Irish seaweeds would benefit food producers and consumers alike. Therefore, the objective of this study was to assess the suitability of brown (sea spaghetti and Irish wakame) and red (dulse and nori) seaweed species harvested from the coast of Ireland as potential health-promoting techno-functional ingredients for use in food products. Seaweeds were characterised based on their nutritional, bioactive (antioxidant), thermogravimetric and technological properties.

3.2. Materials and Methods

3.2.1. Reagents and chemicals

Folin-Ciocalteu reagent, sodium carbonate(Na_2CO_3), gallic acid [$(HO)_3C_6H_2CO_2H$], 2,2-Diphenyl-1-picrylhydrazyl (DPPH), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) ($C_{14}H_{18}O_4$), sodium chloride (NaCl), acetone (CH_3COCH_3), hydrogen peroxide (H_2O_2), boric acid (H_3BO_3), hydrochloric acid (HCl), chloroform ($CHCl_3$), Sulphuric acid (H_2SO_4), methanol (CH_3OH), 2,4,6-Tris(2-pyridyl)-*s*-triazine

(TPTZ) ($C_{18}H_{12}N_6$), ethanol (CH_3CH_2OH), glacial acetic acid (CH_3CO_2H), sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$) and sodium azide (NaN_3) were supplied by Sigma-Aldrich Ireland Ltd., Vale Road, Arklow, Wicklow, Ireland. A dietary fibre enzyme kit, β-glucan (barley), casein, and high-amylose maise starch, were purchased from Megazyme, Bray, Co., Wicklow, Ireland. Silver nitrate and potassium chromate (K_2CrO_4) were purchased from Fischer Scientific, United Kingdom. Potassium dichromate ($K_2Cr_2O_7$) and ferric chloride hexahydrate ($FeCl_3.6H_2O$) were supplied by BDH Limited Supplies, Poole, England. All solvents were of analytical reagent grade.

3.2.2. Seaweed species

Brown (*Himanthalia elongata* - sea spaghetti, *Alaria esculenta* - Irish wakame) and red (*Palmaria palmata*- dulse*, Porphyra umbilicalis* - nori) seaweeds harvested off the coast of Co. Clare in Ireland were obtained from a commercial supplier (Wild Irish Seaweed, Co. Clare, Ireland). All seaweeds were 100% naturally grown, organically certified, air-dried (semi-dried), dehumidified and milled prior to purchase. Milled packaged seaweeds were stored at 4 ^oC until required for analyses. The average particle size of brown and red seaweeds was approximately 2.5 mm (Tyuftin *et al*., 2021).

3.2.3. Nutritional composition

3.2.3.1. Proximate composition

Protein (kjeldahl) using nitrogen-to-protein conversion factor of 5 (AOAC, 1999), fat (soxhlet with chloroform and methanol (2:1)), moisture (oven at 135 ^oC for 2 hr) and ash (muffle furnace at 550 ^oC) contents were analysed by AOAC Methods 954.01,

920.39, 930.15 and 942.05, respectively (AOAC, 1999). Results were expressed as a percentage of the dry seaweed weight.

3.2.3.2. pH and salt

Seaweeds (5 g) were homogenised for 1 min at 14,000 rpm in 45 ml of distilled water using an Ultra Turrax T25 homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen, Germany). The pH of seaweeds was measured at room temperature using a pH meter (Mettler- Toledo, GmbH, Schwerzenbach, Switzerland) by direct insertion of the glass probe.

The salt content of seaweeds (ashed in a muffle furnace as described in section 2.3.1.) was determined by titration using silver nitrate ($AgNO_3$) (Kirk and Sawyer, 1991). The % salt present in seaweed samples was calculated as follows:

$$\% \text{ salt} = \frac{\text{Titre for sample(ml)} - \text{Titre for blank (ml)}}{\text{Mass of sample (g)}} \times \text{Molarity of } AgNO_3 \times 5.844$$

3.2.3.3. Dietary fibre

The total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) (AOAC Method 991.43 and AACC Method 32-07.01) contents were determined using an enzyme kit (Megazyme, Bray, Co. Wicklow, Ireland). β-glucan (barley), casein, and high-amylose maise starch were used as controls to ensure enzyme purity. Results were expressed as a percentage of the dry seaweed weight.

3.2.3.4. Amino acid and mineral contents

The amino acid profile and mineral content of seaweed samples were determined by HPLC with fluorescence detection and pre-column derivatisation (OPA- Ortho Phthalaldehyde derivative) and multi-element inductively coupled plasma-emission spectrometry (ICP-OES), respectively. Analysis was carried out by an independent accredited analytical testing facility (ALS laboratories, Little Island, Cork). Specific details of the analytical methodologies used by the commercial testing facility were unavailable and confidential.

3.2.4. Total phenolic content and in vitro antioxidant activity

3.2.4.1. Preparation of polyphenol-rich extracts from ground seaweeds

Polyphenol rich extracts were prepared as described by Jiménez-Escrig *et al*. (2001) with slight modifications. 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 hr at 20 ^{o}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, and extracts were characterised using the total phenol content (TPC), DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) assays.

3.2.4.2. Total phenol content (TPC)

The total phenol content of the seaweed extracts was measured using the Folin-Ciocalteu method described by Singleton and Rossi (1965) with slight modifications. The extracts of each seaweed were further diluted 1:2 with a mixture of methanol/water (50:50) and acetone/water (70:30), in equal parts, for the assay. Absorbance measurements were recorded at 750 nm using a UV-vis spectrophotometer (Cary 300 Bio, Varian Instruments, CA, USA) against a blank containing all reagents and 0.5 ml of the extract solvents. A gallic acid (0.02- 0.1 mg/ml) standard curve was prepared in the extract solvents, and results were expressed as milligrams of gallic acid equivalents (GAE)/gram of seaweed.

3.2.4.3. DPPH free radical scavenging activity (RSA)

The DPPH free radical scavenging activity of the seaweed extracts was determined as described by Yen and Wu (1999) with slight modifications. Absorbance measurements were recorded at 517 nm using a spectrophotometer (Cary 300 Bio) against a methanol blank. An assay blank containing 2 ml of 0.2 mM DPPH and 2 ml of the extract solvents was prepared for calculation purposes. A standard curve of methanolic Trolox (0.01- 0.04 mg/ml) was prepared, and results were expressed as milligrams of Trolox equivalent (TE)/gram seaweed.

3.2.4.4. Ferric reducing antioxidant power (FRAP)

Total antioxidant activity was measured using a modified FRAP method of Benzie and Strain (1999) with slight modifications. Absorbance measurements were recorded at

593 nm using a spectrophotometer (Cary 300 Bio) against a blank containing all reagents and 0.15 ml of the extract solvents. A standard curve of methanolic Trolox (0.033 - 0.1 mg/ml) was prepared, and results were expressed as milligrams of Trolox equivalent (TE)/gram of seaweed.

3.2.5. Thermal activity

Thermogravimetric analysis (TGA) was carried out using platinum pans on a thermal analyser (Thermal Analysis Q500, TA Instruments, New Castle, DE, USA) using nitrogen gas at a flow rate of 60 ml min^{-1}. Seaweed samples (10 mg) were heated at a rate of 10 ^{o}C min^{-1} with initial and final temperatures of 35 ^{o}C and 900 ^{o}C, respectively. TGA thermograms were analysed using 178 Universal Analysis 2000 software (TA Instruments Ltd., United Kingdom), and results were expressed as % weight loss as a function of increasing temperature.

3.2.6. Technological properties

The water holding, oil holding and swelling capacities of seaweeds were carried out according to Gómez-Ordóñez *et al*. (2010) with modifications.

3.2.6.1. Water holding capacity (WHC)

Distilled water (20 ml) containing 0.02% sodium azide was added to 0.2 g of seaweed samples in filter paper and stoppered funnels. Samples were allowed to stand for 18 h at 4 ^{o}C. Stoppers were removed from the funnels, the residues were weighed, and WHC calculated as g of water retained /g of dry sample.

3.2.6.2. Oil holding capacity (OHC)

Commercial virgin olive oil (10 ml) was added to 0.2 g of seaweed samples in 15 ml centrifuge tubes. Samples were stirred using a magnetic stirrer and allowed to stand at room temperature for 18 h. Following centrifugation at 3,000 × *g* for 20 min, the supernatant was discarded, the residue was weighed, and OHC calculated as g olive oil retained/g of dry sample.

3.2.6.3. Swelling capacity (SC)

Seaweed samples (0.5 g) were weighed into 15 ml measuring cylinders (0.1 ml graduations), and 10 ml distilled water containing 0.02% sodium azide was added. Samples were stirred gently to remove air bubbles and placed on a level surface at room temperature for 18 h. Following the 18 h storage period, the volume (ml) occupied by the samples was measured, and SC was expressed as ml occupied/ g of dry sample.

3.2.7. Statistical analysis

Experiments were analysed on three different batches and in triplicate of each batch. The mean of each batch was analysed. Statistical analysis was performed using the IBM SPSS statistics 25 for windows (SPSS, Chicago, IL, USA) software package. One-way ANOVA was used to examine any significant differences between the means at a 5% significance level. Tukey's post hoc test was used to adjust for multiple comparisons between sample means.

3.3. Results and Discussion

3.3.1. Proximate composition, salt content and pH

Irish wakame possessed higher levels ($P<0.05$) of protein, fat, salt and pH values compared to sea spaghetti (Table 3.1). Stévant *et al*. (2017) reported protein (10.2%) and ash (25.6%) contents for Irish wakame comparable to those in the present study. Cofrades *et al*. (2010) reported similar protein (5%), fat (< 1.5%), and ash (30%) contents for sea spaghetti. In addition, Fernández-Segovia *et al*. (2018) reported a low protein content of 6.8% for sea spaghetti. A higher level of protein (14.08%) has also been reported previously for sea spaghetti, thus highlighting variability in composition associated with seaweed species (Taboada *et al*., 2013).

In the red seaweeds, nori contained significantly higher protein levels compared to dulse. However, dulse had higher ($P<0.05$) moisture, ash, fat, salt contents and pH values than the nori seaweed. Cofrades *et al*. (2010) also reported protein (39%), fat (< 1.5%) and ash (12%) contents for nori, similar to levels reported in Table 3.1. By contrast, Taboada *et al*. (2013) reported a higher fat level (2.8%) for nori composed of 26.6% monounsaturated and 26.9% polyunsaturated fatty acids (23.9% w3 and 2.6% w6) and 43.2% saturated fatty acids. In the present study, red seaweeds contained higher protein levels (~19- 32%) compared to brown seaweeds (~5.6-10.2%), similar to those observed by Fernández-Segovia *et al*. (2018). Phycobiliproteins are pigment proteins in red seaweed species, constituting up to 50% of their total protein content (Niu *et al*., 2007). The high protein content of red seaweed species is comparable to that of terrestrial plant species consumed as a significant source of protein (Taboada *et al*., 2013). For example, red seaweed species such as *Porphyra tenera* (nori) and *Palmaria palmata* (dulse) protein contents have

been compared to that of soybean (Swarnalatha, 2018). The fat content of seaweeds is typically low (1- 3%), with a PUFA content similar to terrestrial vegetables (Swarnalatha, 2018). However, Gressler *et al*. (2011) reported higher fat contents (3.6% and 6.0%) in red seaweeds (*Plocamium brasiliense* and *Ochtodes secundiramea*, respectively).

The pH of seaweeds ranged from 5.55 - 6.71 in brown and red seaweed species (Table 3.1). Abdullah *et al*. (Nurjanah *et al*., 2018) reported pH values of brown seaweed *Sargassum sp.* and red seaweed *E. cottonii* as 6.91 and 6.57, respectively.

Table 3.1. Proximate composition, salt content and pH of brown and red seaweed species.

Seaweed Type		Protein (%)	Moisture (%)	Ash (%)	Fat (%)	Salt (%)	pH
Brown	Sea spaghetti	5.57±0.22[a]	16.31±0.34[a]	30.90±0.44[a]	1.19±0.12[ac]	12.68±0.21[a]	5.55±0.03[a]
	Irish wakame	10.21±0.36[b]	11.79±0.12[b]	30.06±0.28[b]	2.03±0.10[b]	15.30±0.32[b]	6.29±0.01[b]
Red	Dulse	19.04±0.19[c]	10.04±0.41[c]	25.63±0.67[c]	1.52±0.12[a]	14.70±0.95[ab]	6.71±0.01[c]
	Nori	32.03±1.09[d]	8.99±0.16[d]	17.24±0.22[d]	1.02±0.02[c]	5.35±0.12[c]	6.12±0.01[d]

[abcd] % (DW) Mean values (± standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

3.3.2. Dietary fibre content

Total, insoluble (IDF) and soluble (SDF) dietary fibre contents ranged from 31.49 - 49.13%, 22.02 - 37.72% and 3.06 - 12.88%, respectively (Table 3.2). In the brown seaweeds, sea spaghetti contained higher ($P<0.05$) TDF and SDF contents than in Irish wakame, with no significant difference in their IDF contents. Similar TDF levels for sea spaghetti (50.3%) were observed by Cofrades *et al*. (2010). However, Gómez-Ordóñez *et al*. (2010) reported higher SDF (23.63%) and lower IDF (13.51%) in Spanish sea spaghetti, indicative of variation in seaweed nutritional composition, which may be due to their maturity, environmental growth conditions, seasonal period and geographical harvest location (Ortiz *et al*., 2006). In red seaweeds, dulse had a higher ($P<0.05$) SDF content than in nori. However, there was no significant difference in their TDF and IDF contents (Table 3.2). Cofrades *et al*. (2010) also observed comparable TDF levels for nori (35%). Brown seaweed species contained higher ($P<0.05$) TDF and IDF compared to red seaweed species, most probably due to the presence of uronic acids from alginate in brown seaweed species, which contributes to IDF of brown seaweeds (Rupérez and Saura-Calixto, 2001).

From a nutritional perspective, dietary fibres can increase satiety and aid digestive passage through their bulking capacity (Brownlee *et al*., 2005). Seaweeds have a lower carbohydrate content than terrestrial foods, the majority of which is dietary fibre. Terrestrial foods contained approximately 1 - 27% dietary fibre; however, seaweed species can have dietary fibre levels as high as 50 - 93% (Swarnalatha, 2018). Flynn *et al*. (2011) reported the mean daily intake of dietary fibre in Irish adults as 19 g, however the daily recommended intake of dietary fibre is 25 - 35 g/day (Stephen *et al*., 2017). To compare the probable contribution of seaweeds to diet, 8 g DW of

seaweed was used as a serving size. Seaweed species examined in this present study, where TDF ranged from ~31.4 - 49%, can provide 10.5 - 16.4% of the daily dietary fibre requirement in an 8 g serving.

Table 3.2. Composition of dietary fibre in brown and red seaweed species.

Seaweed Type		TDF (%)	IDF (%)	SDF (%)
Brown	Sea spaghetti	49.13±0.92[a]	37.49±0.89[a]	11.65±0.38[a]
	Irish wakame	40.78±1.57[b]	37.72±1.13[a]	3.06±1.03[b]
Red	Dulse	34.89±2.93[c]	22.02±3.33[b]	12.88±1.33[a]
	Nori	31.49±1.83[c]	23.61±1.28[b]	7.88±0.56[c]

[abc] % (DW) Mean values (± standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

3.3.3. Amino acid content

In the brown seaweeds, Irish wakame had higher (P<0.05) essential amino acids (threonine, valine, iso-leucine and leucine) and all non-essential amino acids, except tyrosine, in comparison to sea spaghetti (Table 3.3). Irish wakame also had higher (P<0.05) total EAA (essential amino acid), total non-EAA and total AA contents than in sea spaghetti. Total EAA, total non-EAA, and total AA contents of Irish wakame were approximately double that of sea spaghetti (Table 3.3). Cofrades *et al*. (2010) reported similar EAA and Total AA contents for sea spaghetti (1.96% and 4.4%, respectively).

Nori had significantly higher essential amino acids (threonine, valine, lysine, iso-leucine, leucine and phenylalanine) and all non-essential amino acids, except cysteine and tyrosine, than in dulse. Nori exhibited higher (P<0.05) total EAA, total non-EAA and total AA contents compared to dulse. Total EAA, total non-EAA, and

total AA contents of nori were also approximately double that of dulse (Table 3.3). Cofrades *et al*. (2010) also reported comparable EAA and Total AA contents for nori (13.44% and 36.45%, respectively).

Red seaweed species contained higher ($P<0.05$) levels of each amino acid type (excluding histidine, methionine, phenylalanine, cystine and tyrosine) compared to the brown seaweed species (Table 3.3), this can be attributed to the significantly higher protein levels present in red seaweed species (Table 3.1). The total AA contents are comparable with the protein content of each seaweed species presented in Table 3.1.

In this study, aspartic and glutamic acids were the most abundantly occurring non-essential amino acids in seaweed species, as observed previously by Gressler *et al*. (2011). Mabeau *et al*. (1992) reported that the high levels of aspartic and glutamic acids in seaweeds are responsible for their distinct flavour (umami flavour) and taste. Mišurcová *et al*. (2014) also indicated that alanine, glycine and glutamic acid were considered the main components of seaweed flavour.

Table 3.3. The amino acid content (%) of brown and red seaweed species.

Amino acids		Brown seaweed		Red seaweed	
		Sea spaghetti	Irish wakame	Dulse	Nori
Essential	Histidine	0.09±0.00^{a}	0.13±0.06^{a}	0.20±0.15^{ab}	0.47±0.01^{b}
	Threonine	0.23±0.00^{a}	0.50±0.01^{b}	0.84±0.02^{c}	1.75±0.09^{d}
	Valine	0.25±0.01^{a}	0.53±0.01^{b}	0.98±0.02^{c}	1.70±0.08^{d}
	Methionine	0.14±0.05^{a}	0.13±0.15^{a}	0.20±0.23^{a}	0.29±0.35^{a}
	Lysine	0.31±0.03^{a}	0.57±0.02^{a}	1.19±0.07^{b}	1.63±0.16^{c}
	Iso-leucine	0.20±0.01^{a}	0.39±0.00^{b}	0.62±0.00^{c}	1.06±0.09^{d}
	Leucine	0.35±0.01^{a}	0.74±0.01^{b}	1.16±0.03^{c}	2.20±0.00^{d}

	Phenylalanine	0.30±0.07[a]	0.51±0.00[ab]	0.73±0.07[b]	1.25±0.06[c]
Total EAA		1.86±0.19[a]	3.51±0.22[b]	5.92±0.31[c]	10.36± 0.55[d]
Non-Essential	Aspartic	0.48±0.02[a]	1.11±0.00[b]	1.90±0.01[c]	3.41±0.00[d]
	Serine	0.27±0.00[a]	0.56±0.02[b]	1.09±0.00[c]	1.72±0.01[d]
	Glutamic	0.59±0.01[a]	1.32±0.15[b]	2.01±0.17[c]	4.15±0.06[d]
	Glycine	0.27±0.01[a]	0.59±0.01[b]	1.13±0.02[c]	2.02±0.01[d]
	Arginine	0.25±0.01[a]	0.49±0.03[b]	1.07±0.06[c]	3.73±0.09[d]
	Alanine	0.33±0.01[a]	0.93±0.10[b]	1.36±0.02[c]	3.90±0.01[d]
	Proline	0.20±0.01[a]	0.42±0.02[b]	0.89±0.10[c]	1.30±0.01[d]
	Cystine	0.07±0.00[a]	0.26±0.00[b]	0.34±0.02[c]	0.24±0.01[b]
	Tyrosine	0.12±0.00[a]	0.33±0.00[ab]	0.34±0.42[ab]	0.99±0.01[b]
Total Non-EAA		2.58± 0.06[a]	6.01±0.33[b]	10.13±0.76[c]	21.44±0.16[d]
Total AA		4.44±0.25[a]	9.52±0.55[b]	16.05±1.07[c]	31.80±0.71[d]

[abcd] % (DW) Mean values (± standard deviation) in the same row bearing different superscripts are significantly different (P<0.05).

3.3.4. Mineral content

Macro (Sodium (Na), Potassium (K), Phosphorus (P), Magnesium (Mg) and Calcium (Ca)) and trace mineral (Iron (Fe), Manganese (Mn), Zinc (Zn) and Copper (Cu)) contents, nutritional intake from an 8 g serving and mineral reference nutrient intake (RNI) levels for children and adults are presented in Table 3.4. In general, seaweeds contain high levels of minerals due to their capacity to absorb and store inorganic substances from the marine environment (Rupérez and Saura-Calixto, 2001). Sea spaghetti had higher Na and Mg contents than Irish wakame, and nori had higher P, Mg, Zn and Cu contents than dulse. Furthermore, brown seaweeds had higher Na, Mg and Ca contents than red seaweed species in this study. Similar findings were reported by Cofrades *et al*. (2010).

Na and K were the most abundant in all seaweed species, consistent with a previously reported study by Cofrades *et al*. (2010). Na is an essential mineral in the human body; however, excess consumption can be detrimental to human health. A sodium intake of less than 2000 mg per day is recommended (WHO, 2012). Recently, Morrissey *et al*. (2020) reported a mean dietary sodium intake in Ireland of 2877 mg/day in men and 2134 mg/day in women. Seaweeds contain high concentrations of K (higher than in terrestrial fruits and vegetables), which at high intake levels (≤ 3510 mg is recommended daily (WHO, 2012) has been reported to protect against high blood pressure and other cardiovascular diseases (Preuss *et al*., 2003). It has been reported that the ratio of sodium to potassium (≤ 1.0) in the diet may be more significant than the amount of each mineral (Wijendran and Bell, 2019). The ratio of sodium to potassium (Na:K) in the seaweed species examined was low (Irish wakame- 0.74, dulse- 0.32 and dulse- 0.49), which helps combat fluid retention and high blood pressure without the risk of impacting the potassium balance (Rupérez, 2002). However, sea spaghetti had a high Na/K ratio of 1.94 and sources of potassium should be consumed when consuming this seaweed to balance the Na/K ratio.

The phosphorus content of seaweeds ranged from 77.7- 498 mg/ 100 g seaweed. This mineral is required in the body in association with calcium. Seaweed species also contained high Mg and Ca contents. Magnesium deficiency in humans is common and has been linked to chronic diseases (MacArtain *et al*., 2007). Seaweed species in this study can contribute 8.6% - 26.3% of Mg RNI of adults in an 8 g serving (Table 3.4). Seaweeds in this study contain higher Ca levels than present in bovine milk (120 mg/ 100 g) and spinach (145 mg/ 100 g) (Swarnalatha, 2018).

Dulse had the highest Fe and Mn contents compared to the other trace elements and seaweed species examined (Table 3.4). MacArtain *et al*. (2007) concluded that specific seaweeds have higher Fe content than terrestrial sources of Fe such as spinach and meat. Iron deficiency is one of the most prevalent nutritional deficiencies worldwide in developing and developed nations (Neumann *et al*., 2002). Seaweed species in this study can contribute 1.8% - 27.2% of iron RNI of children and adults in an 8 g serving (Table 3.4). Mn is also an essential nutrient involved in the processing of cholesterol, carbohydrates, and protein in the body. The high Mn levels present in dulse (49.1 mg/100 g) results in the provision of approximately 4 mg Mn in an 8 g serving. This exceeds the safe intake values suggested by the Committee on Medical Aspects of Food and Nutrition Policy (1991) ; however, a 2.6 g serving will provide approximately 1.3 mg, which is regarded as safe for an adult. The Zn content in seaweed species ranged from 0.94- 6.12 mg/ 100 g seaweed. The reference nutrient intake of Cu per day is 1.2 mg (adults), and seaweed species in this study can contribute up to 3% of the RNI in an 8 g serving (Table 3.4). Other sources of Cu in the human diet include meat and vegetable.

On the other hand, seaweeds can also possess toxic elements such as arsenic (As) and cadmium (Cd), with potentially negative effects on human health. The form of arsenic which is usually of concern is its toxic inorganic form (iAS) which is a known human carcinogen associated with liver, bladder, lung and skin cancers (Hughes, 2002). Majority of arsenic in seaweed appear to be in the form of less toxic arsenic forms (FSAI, 2009). Currently, the EU have no limits for arsenic levels in foods, however, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) provisional tolerable daily intake (PTDI) for inorganic arsenic is 0.002 mg/kg

bodyweight, equivalent to 0.12 mg/day for a 60kg adult (FSAI, 2009). From previous studies, sea spaghetti, Irish wakame, dulse and nori contain 0.202 mg/kg, 0.220 mg/kg, 0.466 mg/kg and 0.239 mg/kg of arsenic, respectively (Almela *et al*., 2006; Stévant *et al*., 2018). The maximum level of cadmium in foods consisting exclusively or mainly of dried seaweed or of products derived from seaweed is 3.0 mg/kg of seaweed or seaweed product (FSAI, 2009). Sea spaghetti, Irish wakame, dulse and nori contain 0.222-0.395 mg/kg, 1.55-2.01 mg/kg, 0.147 mg/kg and 0.126 mg/kg of cadmium, respectively (Almela *et al*., 2006; Stévant *et al*., 2018).

Table 3.4. The mineral content of brown and red seaweed species (mg/100g) and RNI (mg/day) of each mineral type.

Minerals		Mineral levels [$]				Nutritional intake (8 g serving)				RNI [#]	
		Brown seaweeds		Red seaweeds		Brown seaweeds		Red seaweeds		Infants and children	Adults
		Sea spaghetti	Irish wakame	Dulse	Nori	Sea spaghetti	Irish wakame	Dulse	Nori		
Macro	Na	5419±3.61[a]	3920±4.73[b]	2080±0.29[c]	1710±4.86[d]	433.60	313.60	166.40	136.80	210 - 1600	1600
	K	2791±7.21[a]	5331±2.65[b]	6440±1.80[c]	3460±4.75[d]	223.20	426.40	515.20	276.80	800 - 3100	3500
	P	77.70±0.65[a]	311±2.31[b]	354±0.76[c]	498±1.04[d]	6.22	24.88	28.32	39.84	270 - 770	540
	Mg	985±2.50[a]	797±1.76[b]	322±1.50[c]	363±3.51[d]	78.80	63.76	25.76	29.04	55 - 300	270 - 300
	Ca	957±3.04[a]	986±1.53[b]	282±3.61[c]	152±2.65[d]	76.56	78.88	22.56	12.16	525 - 750	700
Na: K		1.94	0.74	0.32	0.49						
Trace	Fe	1.96±0.03[a]	8.74±0.03[b]	50.30±0.72[c]	20.20±1.04[d]	0.16	0.70	4.02	1.62	8.7 - 14.8	1.7 - 14.8
	Mn	2.49±0.04[a]	0.986±0.00[b]	49.13±0.32[c]	4.43±0.09[d]	0.20	0.08	3.93	0.35	0.016*	1.4*
	Zn	0.94±0.00[a]	2.21±0.04[b]	3.93±0.02[c]	6.12±0.03[d]	0.08	0.18	0.31	0.49	4.0 - 9	7.0 - 9.5
	Cu	0.065±0.01[a]	0.109±0.00[a]	0.40±0.00[b]	0.547±0.06[c]	0.005	0.009	0.03	0.04	0.3 - 1.0	1.2

[abc] mg/100g Mean values (± standard deviation) in the same row bearing different superscripts are significantly different ($P<0.05$) [$]

Na: K ratio- dimensionless

[#] RNI - reference nutrient intake in mg/day (Committee on Medical Aspects of Food and Nutrition Policy (1991)).

[*] Safe Intakes

3.3.5. Total phenolic content and in vitro antioxidant activity

A range of compound classes present in seaweeds (carotenoids, phenolic and polyphenolic compounds, pigments, sulphated polysaccharides and vitamins) exhibit varying degrees of antioxidant activity. The level of antioxidant activity or potency associated with a particular seaweed type is influenced by factors such as seasonality, species (red, green or brown seaweeds) and solvents used during the extraction process, i.e. extract manufacture. In the present study, the total phenolic content (TPC) and in vitro antioxidant activities (DPPH and FRAP) followed the order: sea spaghetti (brown) ≥ nori (red) > Irish wakame (brown) > dulse (red) ($P<0.05$) (Figure 3.1).

In brown seaweeds, sea spaghetti had higher ($P<0.05$) TPC, DPPH and FRAP activities than Irish wakame. Comparable TPC (18 mg GAE/ g seaweed) and FRAP activity (4.7-11.7 mg TE/g seaweed) levels in sea spaghetti has been previously reported in the scientific literature (Fernández-Segovia *et al*., 2018; Rajauria and Abu-Ghannam, 2013). Phlorotannins (composed of phloroglucinol sub-units) are unique to brown seaweeds and represent the major class of polyphenolic compounds present. Ummat *et al*. (2020) reported higher TPC, total phlorotannin and flavonoid levels, DPPH and FRAP activities in sea spaghetti than in Irish wakame, similar to findings reported in the current study (Figure 3.1). The magnitude of difference between sea spaghetti and Irish wakame DPPH activities was lower than observed for the TPC activity. This may be attributed to the presence of antioxidant compounds such as fucoxanthin, which, has been previously reported at higher levels in Irish wakame (0.8 – 0.9 mg/g) than in sea spaghetti (0.3- 0.4 mg/g) (Shannon and Abu-Ghannam, 2017). In the

absence of a detailed chemical characterisation of the antioxidant compounds present in each seaweed, using for example, liquid chromatography mass spectrometry analytical techniques, differences in DPPH and FRAP activities were attributed to compositional variations in the profile of antioxidant compounds contained in the brown seaweed species examined.

In red seaweeds, nori had higher ($P<0.05$) TPC, DPPH and FRAP activities compared to dulse (Figure 3.1). Similarly, (Ferraces-Casais *et al*., 2012) also reported a higher TPC for nori compared to dulse. Phenolic compound classes present in red seaweeds include bromophenols, flavonoids, phenolics acids, phenolic terpenoids, and mycosporine-like amino acids (MAAs) (Cotas *et al*., 2020). To date, the scientific literature contains limited information on the identification of such phenolic compounds including those contained in the red species examined in this study. Polyphenolic flavonoid catechin compounds are present in many seaweed species. Rodríguez-Bernaldo de Quirós *et al*. (2010) reported higher levels of catechins and associated catechin isomers (epicatechin (EC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG)) in nori (*Porphyra spp.)* compared to dulse (*Palmaria spp.).* This may, in part, explain the higher TPC levels observed in the nori seaweed. In addition, (Ferraces-Casais *et al*., 2012) reported higher levels of carotenoids (β-carotene, lutein), pigments (chlorophyll a, pheophytin a) and vitamins C (ascorbic acid) and E (tocopherols) in nori compared to dulse, all of which would contribute to the higher ($P<0.05$) DPPH and FRAP activities observed in the nori species.

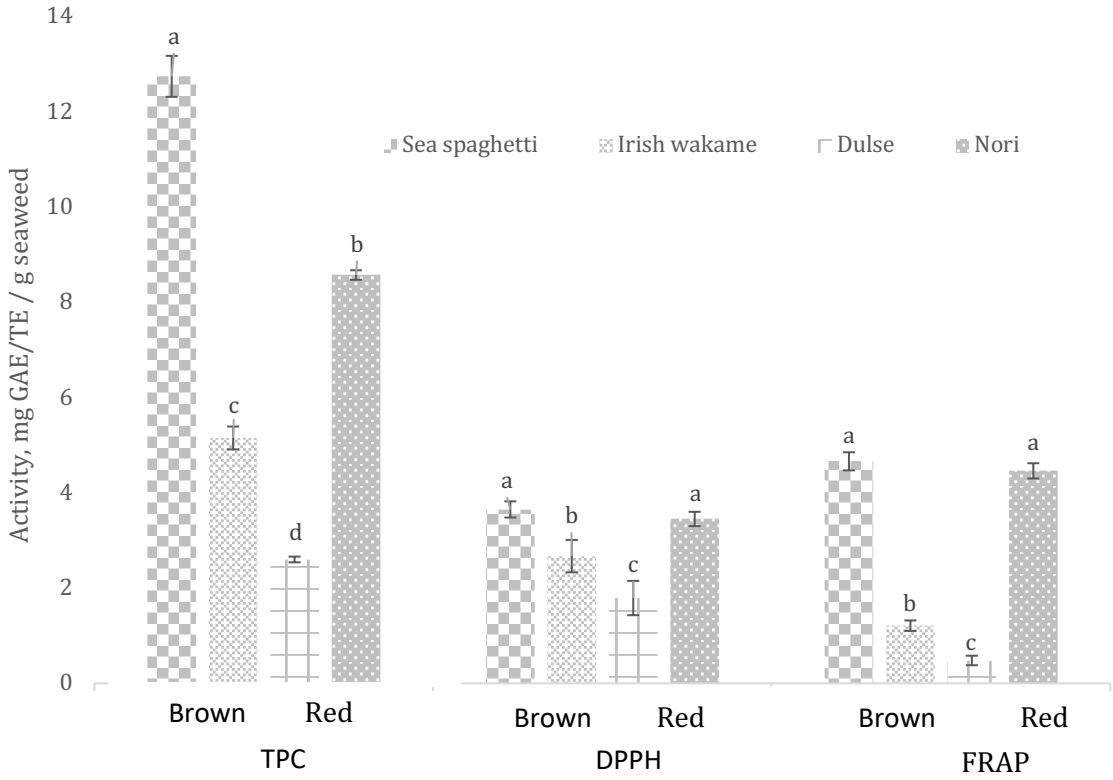


Figure 3.1 Total phenolic content (GAE/g) and *in vitro* antioxidant activity (TE/g) of brown and red seaweed species.

[abc] Mean (± standard deviation) mg GAE/ TE / g seaweed. Within each assay, bars containing different letters are significantly different (P 0.05). GAE = Gallic acid equivalent, mg/g extract (TPC). TE = Trolox equivalent, mg/g extract (DPPH and FRAP)

3.3.6. Thermal activity

The TGA curves of brown and red seaweeds are shown in Figure 3.2. The initial weight loss (35 °C to approximately 200 °C) is due to dehydration (cellular and external water loss). The maximum rate of weight loss occurred at approximately 250 °C. The main degradation of components occurred approximately between 200 °C – 400 °C. Yanik *et al*. (2013) reported the main degradation of seaweeds occurred between 190 °C-390 °C and a maximum weight loss at 260 °C. Ross *et al*. (2008) also reported that

carbohydrate decomposition occurred in a range of 180 °C – 270 °C and protein at 320 °C – 450 °C. At 550 °C, the mass loss of sea spaghetti and Irish wakame was approximately 55% and 57%, respectively. At 900 °C, approximately 35% of components were present in brown seaweed species, which may decompose at a higher temperature.

At 550 °C, the mass loss of dulse and nori were approximately 60% and 65%, and at 900 °C, about 30% and 24% of components were present in dulse and nori, respectively. Results indicated that organic nutritional components (carbohydrates) in seaweeds and seaweed food products cannot withstand processing temperatures greater than 250 °C. Seaweed composition varies; therefore, the degradation behaviour may also vary.

At 900 °C, the high inorganic residue content of these seaweed species is possibly due to their high mineral content (Table 3.4) and other unknown components which may decompose at a high temperature (> 900 °C). These minerals and components may be difficult to pyrolyze and therefore pyrolyze at slow rates; most likely representing constituents of the final weight. Weight loss (%) at 900 °C followed the same trend as the results for ash analysis: sea spaghetti ≥ Irish wakame > dulse > nori (Table 3.1).

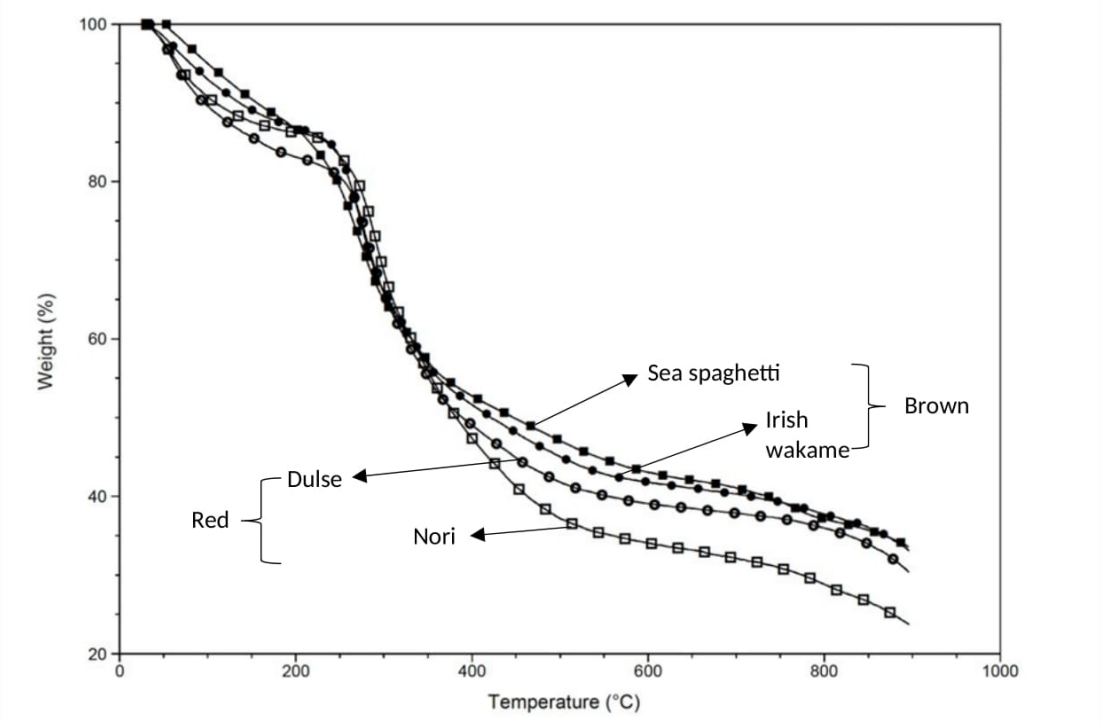


Figure 3.2 Thermogravimetric analysis curves of brown and red seaweed species under a nitrogen atmosphere.

3.3.7. Technological properties

The water holding capacity (WHC), oil holding capacity (OHC) and swelling capacity (SC) data for brown seaweeds indicated no significant differences ($P<0.05$) between sea spaghetti and Irish wakame (Table 3.5). Gómez-Ordóñez *et al*. (2010) reported similar WHC (7.26 g/ g dry weight) and OHC (1.61 g/ g dry weight), and slightly higher SC (10.97 ml/ g dry weight) for sea spaghetti. In the red seaweeds, nori had higher ($P<0.05$) WHC and SC than dulse. Nori also had higher OHC than dulse, however not significant ($P>0.05$). Dulse had the lowest ($P<0.05$) WHC and SC than the other seaweeds examined in this study (Table 3.5). Rupérez and Saura-Calixto (2001) reported similar WHC (5.12 g/ g dry weight), and lower SC (6.08 ml/ g dry weight) and

OHC (1.04 g/ g dry weight) for nori. In this study, brown seaweed species had lower ($P<0.05$) OHC than red seaweed species.

The seaweed species examined in this research had high total dietary fibre contents (>30% DW) and, combined with the protein contents (Table 3.1), represent approximately 40- 55% DW. Wong and Cheung (2000) and Yaich *et al*. (2011) suggested that the total dietary fibre, type of polysaccharides (alginate, agar and carrageenan), protein content and proteins present in the cell wall may have an impact on the technological properties (WHC, OHC and SC) of seaweed species. Other than the structure of fibres, technological properties can also be affected by factors such as temperature, particle size, porosity, pH, ionic strength, types of ions in solutions, and density are also important to understand the different behaviours of samples during hydration (Fleury and Lahaye, 1991). Factors listed above or a combination of factors may be responsible for the lower WHC in dulse and higher SC in nori. Wong and Cheung (2000) and Yaich *et al*. (2011) also proposed that the mechanism of OHC of seaweeds is mainly due to the physical entrapment of oil by capillary attraction and the hydrophobicity of proteins. Also, the variation in OHC amongst seaweed species may be due to the different proportions of polar side chains of the amino acids on the surfaces of protein molecules. The red seaweed species examined in this study contained higher protein levels than brown seaweed species (Table 3.1), which explains their higher capacity to hold oil.

Table 3.5. Technological properties (WHC (g/g), OHC (g/g) and SC (ml/g)) of brown and red seaweeds species.

Seaweed Type		WHC (g water held/g dry sample)	OHC (g olive oil retained/g sample)	SC (ml occupied by sample/g dry sample)
Brown	Sea spaghetti	6.91±0.57[a]	1.37±0.05[a]	7.50±0.08[a]
	Irish wakame	6.69±0.37[a]	1.73±0.17[a]	7.95±0.13[a]
Red	Dulse	3.76±0.23[b]	2.90±0.50[b]	6.30±0.15[b]
	Nori	6.89±0.42[a]	3.59±0.13[b]	13.22±0.45[c]

[abc] Mean values in the same column bearing different superscripts are significantly different ($P<0.05$).

3.4. Conclusions

Edible and commercially available Irish brown (*Himanthalia elongata*- sea spaghetti and *Alaria esculenta*- Irish wakame) and red (*Palmaria palmata*- dulse, *Porphyra umbilicalis*- nori) seaweed species represent potential nutritional sources of protein, amino acids, dietary fibre, minerals and health–promoting bioactive antioxidant compounds in the human diet. Seaweed species in this study contained low fat levels, thereby reducing their caloric content when ingested alone or as a component of a food product. Brown seaweed species are significant sources of minerals (Na, Mg and Ca), total and insoluble dietary fibres, while red seaweed species possessed superiority with respect to their protein content, phosphorus, and trace mineral levels. Sea spaghetti and nori displayed the highest TPC and *in vitro* antioxidant activity indicating suitability of these seaweed species as superior sources of bioactive compounds in the diet or as ingredients for use in the manufacture of functional food products. From a food processing and technological perspective, seaweeds demonstrated thermal stability at a high temperature and varying degrees of water, oil and swelling capacities, which would ultimately influence the textural properties of food products. Further research is necessary to explore the use of commercially available and sustainable Irish seaweed species as, health-promoting functional ingredients in various food systems such as processed foods, meat products, vegan foods and high protein meals.

CHAPTER 4.

Determination of acceptable inclusion levels for selected brown and red Irish seaweed species in pork sausages

Abstract

Commercially available Irish edible brown (*Himanthalia elongata* - sea spaghetti (SS), *Alaria esculenta* - Irish wakame (IW)) and red seaweeds (*Palmaria palmata* – dulse (PP), *Porphyra umbilicalis* - nori) were incorporated into pork sausages at 1% DW, 2.5% DW and 5% DW. Proximate composition, salt, water holding (WHC), cook loss, instrumental colour analysis, texture profile analysis (TPA) and sensory analysis were examined. Protein (13.14 – 15.60%), moisture (52.81 – 55.71%) and fat (18.79 – 20.02%) contents of fresh pork sausages were not influenced ($P > 0.05$) by seaweed type or addition level. The ash content of pork sausages containing PP, SS and IW at 2.5% and 5% and nori at 5%, were higher ($P<0.05$) than the control sample. In comparison to the control, sausages containing nori, SS and IW at 5% displayed higher ($P<0.05$) WHC. Cook loss was unaffected ($P>0.05$) by the addition of seaweeds into sausage formulations, compared to the control and within each seaweed. The addition of seaweeds into sausages had an impact on the surface colour (L*a*b*) and texture profile analysis (TPA) at different inclusion levels. Overall, the maximum acceptable inclusion level for PP is 1% and 2.5% for the other seaweed species examined. Furthermore, the preference for seaweed sausages was ranked in the following order: nori > SS > IW > PP.

4.1. Introduction

Interest in food products that can promote health and well-being has increased within the food industry and amongst consumers. Such foods are generically termed 'functional foods' where health benefits exist beyond basic nutrition (Sloan, 1999). Since ancient times, seaweeds have been consumed as food and utilised as traditional drugs in Asian countries. Recently, seaweeds have gained significance in western countries as a functional food or as ingredients for use in the development of functional food products (Bocanegra *et al*., 2009; Hur, 1999).

Seaweeds contain bioactive compounds and nutritional properties such as high levels of quality protein, minerals, vitamins, dietary fibre, polyphenols, carotenoids and tocopherols (López-López, Cofrades, and Jiménez-Colmenero, 2009). They are also low in fat, and selected species contain prominent levels of n-3 polyunsaturated fatty acids (PUFAs) (Fernández-Martín *et al*., 2009). Bioactivities and health benefits associated with compounds present in seaweed, include antioxidant, anti-inflammatory, anti-coagulation, anti-proliferation and antibiotic properties (Jaswir *et al*., 2014). In addition to enhancing healthiness, seaweeds can also impact the physicochemical and sensory properties of meat products. Seaweed polysaccharides (dietary fibre); alginate from brown seaweeds, and agar and carrageenan from red seaweeds have been extensively utilised by the food industry as texture modifiers due to their high viscosity and gelling properties (Jiménez-Escrig and Sánchez-Muniz, 2000). These textural influencing properties of seaweeds may enhance the texture and acceptability of processed meat products by consumers. For instance, alginate and carrageenan have been used as fat replacers in processed meats to produce

healthier products without compromising on the benefits of fat such as texture and flavour (Cao *et al*., 2021; Cierach *et al*., 2009; Kang *et al*., 2020; Kumar and Sharma, 2004; Qin *et al*., 2020; Totosaus *et al*., 2004). Additionally, agar has also been used as a gelling agent by poultry and meat canners, and as meat substitutes (Selby and Whistler, 1993). Minerals such as sodium and potassium are present in brown and red seaweeds in high amounts and these minerals can present a saltiness flavour and taste in meat products. Prominent levels of aspartic and glutamic amino acids in seaweeds are responsible for their distinctive umami flavour and taste, which may be desirable to consumers and increase their interest to consume seaweed-containing meat products (Mabeau *et al*., 1992).

Seaweeds have been added to processed meat products as functional ingredients without reducing other product components such as fat, salt and phosphate levels, for example, sea spaghetti (*Himanthalia elongata*) in beef patties (Cox and Abu-Ghannam, 2013), sea tangle (Laminaria japonica) in breakfast sausages (Kim *et al*., 2010), sea mustard (*Undaria pinnatifida*), green laver (*Ulva spp*.) and *Sargassum fusiforme* in pork patties (Jeon and Choi, 2012). This study is similar to previous studies in that it too investigates the functionality of selected seaweeds, but it differs significantly in that it does so while using commercially available Irish seaweeds at three different inclusion levels. Additionally, the nutritional composition, antioxidant activity, technological and thermal properties of the selected seaweeds employed in this study have been analysed and quantified. Results from this study concluded that these brown and red seaweed species have nutritional sources of protein, amino acids, dietary fibre, minerals, and health–promoting bioactive antioxidant

compounds and demonstrated thermal stability at high temperature application (Chapter 3).

To the best of our knowledge, seaweed species employed in this study which were harvested from the coast of Ireland have not been extensively explored previously as potential functional ingredients in processed meat products at different inclusion levels. Hence, the objective of this study was to investigate the effect of commercially available brown (*Himanthalia elongata* - sea spaghetti, *Alaria esculenta* - Irish wakame) and red, (*Palmaria palmata* – dulse, *Porphyra umbilicalis* - nori) Irish seaweed species on the physicochemical and sensory properties of pork sausages. Acceptable inclusion levels of these seaweeds from a sensory perspective were determined.

4.2. Materials and Methods

4.2.1. Chemicals and reagents

Sodium chloride (NaCl), hydrogen peroxide (H_2O_2), boric acid (H_3BO_3), hydrochloric acid (HCl) and Sulphuric acid (H_2SO_4) were supplied by Sigma- Aldrich Ireland Ltd., Vale Road, Arklow, Wicklow, Ireland.

4.2.2. Raw materials

Brown *(Himanthalia elongata*- sea spaghetti (SS), *Alaria esculenta*- Irish wakame (IW)) and red seaweed species (*Palmaria palmata*- dulse (PP), *Porphyra umbilicalis*- nori) were used in this research. Seaweeds were bought from Wild Irish Seaweed, Co. Clare, Ireland. According to supplier specification, all seaweeds were hand-

harvested off the coast of Co. Clare, Ireland, 100% naturally grown and sustainably harvested and organically certified. Seaweed species were air-dried, dehumified, and milled prior to purchase. The average particle size of brown and red seaweeds was approximately 2.5 mm (Tyuftin *et al*., 2021).

Pork oyster muscle and pork backfat were supplied by Ballyburden Meat Processors (Cork, Ireland). Sausage seasoning (Salt, sugar, dextrose, stabiliser (E450, E452), flavour enhancer (E621), preservative (E223, sodium metabisulphite), antioxidant (E301), rusk [wheat flour (calcium carbonate, iron, niacin, thiamin), salt], natural flavourings, rapeseed oil, hydrolysed vegetable protein (soya, maise)) with a total of 58.78% salt was obtained from Redbrook Ingredient Services (Mulhuddart, Dublin 15, Ireland). Rusk [Wheat flour (wheat flour, calcium carbonate, Iron, Niacin, Thiamin), salt (1.2%), raising agent E503] and collagen casing were purchased from Kiernans Food Ingredients Ltd. (Dundalk Road, Carrickmacross, Co. Monaghan).

4.2.3. Sausage production

Fresh pork sausages (5 kg batches) were formulated (Table 4.1). Sausages were manufactured by mincing the pork meat and backfat through a 4 mm hole plate (Talsabell SA., Valencia, Spain). Pork oyster, half of the water and the seasoning were mixed for 60 seconds at high speed using a bowl chopper (Seydleman Bowl chopper, Burgstallstraße, Germany). The backfat and various seaweeds were added and mixed at high speed for 30 seconds. The other half of the water was added, and the batter was further mixed for 30 seconds at high speed. Rusk was added and mixed at low speed for 30 seconds. Sausage batter was stuffed using piston-type sausage filler

(Mainca UK Ltd., Berkshire, UK) into 21 mm diameter collagen casings and hand-linked.

Table 4.1. Formulation (%) of pork sausage treatments.

Sample	Control	Seaweed inclusion levels		
		1%	2.5%	5%
Pork oyster	45.00	44.00	42.50	40.00
Pork back fat	20.00	20.00	20.00	20.00
Water	20.00	20.00	20.00	20.00
Rusk	12.50	12.50	12.50	12.50
Seasoning	2.50	2.50	2.50	2.50
Seaweed	0.00	1.00	2.50	5.00

4.2.4. Proximate composition

Protein (Kjeldahl) and ash (muffle furnace at 550 ^{o}C) contents were analysed by AOAC Methods 954.01 and 942.05, respectively (AOAC, 1999). Results were expressed as a g/ 100g (%) of sample. Moisture and fat contents of sausages were determined using a SMART Trac system (CEM GmbH, Kamp- Lintfort, Germany).

4.2.5. Salt analysis

The salt content was determined by titration using silver nitrate (Kirk and Sawyer, 1991). Silver nitrate (0.1 N $AgNO_3$) solution was standardised against 0.1% sodium chloride (NaCl) solution. Samples were placed in a muffle furnace as described in

section 4.2.4. Ash was washed into a conical flask with 20ml distilled water. Then, 2ml of indicator (potassium chromate and potassium dichromate) was added, and standardised silver nitrate was used to titrate the solution from a clear yellow to an opaque light orange. Blank titration was performed using 20ml distilled water. Salt was calculated using the following calculation:

$$\% \text{ salt} = \frac{\text{Titre for sample (ml)} - \text{Titre for blank (ml)}}{\text{Mass of sample (g)}} \times \text{Molarity of } AgNO_3 \times 5.844$$

4.2.6. Water holding capacity (WHC)

The water holding capacity was measured using the method described by (Lianji and Chen, 1989). Fresh sausage samples (10 g) were weighed into glass tubes and heated in a water bath for 10 min at 90 ^{o}C. After heating, the samples were removed, wrapped in cheesecloth, and placed in 30ml centrifuge tubes lined with cotton wool at the bottom of each tube. Samples were centrifuged at 13,300 g for 10 min at 4 ^{o}C. After centrifugation, the cheesecloth was removed, and samples were reweighed. Water holding capacity was calculated using the following calculation:

% Water holding capacity=1-((B-A))/((B×M)) ×100

B is the sample's weight before heating, A is the weight of the sample after heating and centrifuging, and M is the % moisture of the sample.

4.2.7. Cook loss

Sausage weight was recorded before and after cooking. Sausages were cooked in a Zanussi oven at 180 ^{o}C for 22 min, and an internal temperature of 72 ^{o}C was reached. Cook loss was calculated using the following equation:

% Cook loss= Raw weight-cooked weight/Raw weight ×100

4.2.8. Instrumental colour analysis

The colour of fresh sausage samples was measured according to the CIE L*a*b* colour system. The samples were allowed to equilibrate at room temperature for 30 minutes before measuring. The surface colour was measured using a Minolta chromameter (CR400, Minolta Camera Co.Ltd, Osaka, Japan) with an 11mm-diameter aperture and D65 illuminant. The chromameter was calibrated using a white tile (L=97.79, a=-0.11, b= 2.69).

4.2.9. Texture profile analysis (TPA)

The texture profile analysis (TPA) of sausage samples was carried out using a texture analyser TA-XF10S1.5i (Stable Micro System, Surrey, England) based on a method described by Bourne (1978). Cooked sausage samples were allowed to equilibrate at room temperature for 30 minutes before measuring. The samples were cut into cylindrical slices (10 mm thickness), and four pieces were measured per sausage sample. The samples were subjected to a two-cycle compression using a 30 kg load cell. All samples were compressed to 40% of their original height using a cylindrical

probe (SMP/35 compression plate) at a cross-head speed of 1.5 mm/s. Determining factors include; hardness (N), the maximum force required for the first compression; springiness (mm), distance the sample recovers after initial compression; cohesiveness (dimensionless), the ratio of positive force area during the second compression and chewiness (N*mm), the product of gumminess and springiness.

4.2.10. Sensory Acceptance Testing (SAT)

Sausages were cooked as described in 4.2.7 above. Sausages were left to cool to room temperature and cut into cylindrical slices (~2cm) and labelled with a three-digit random number. The Hedonic (liking) test was carried out using an untrained panellist (n=25) who were familiar with pork sausages (Stone *et al*., 2012). Sensory analysis was conducted in sensory booths that conform to international standards (ISO, 2014) at room temperature. Assessors were presented with 13-treatments duplicated in a randomised order over three sessions (first session- 10 samples, second and third sessions- 8 samples per session) to reduce panellist fatigue. Water for palate cleansing between samples was also provided. Assessors were asked to indicate their scores for the following hedonic terms: appearance, aroma, flavour, texture, and overall acceptability on a 10-cm continuous line scale (1= extremely dislike and 10=extremely like). Assessors were also asked to select their most preferred sample. The mean values for each treatment per panellist were analysed.

4.2.11. Statistical analysis

Physiochemical analyses were carried out on three independent trials, on duplicate samples per trial and three readings were taken per sample. Data were presented in triplicate (average per trial). For SAT data, the mean of each panellist was presented (n=25). Statistical analysis was carried out using the IBM SPSS statistics 25 for windows (SPSS, Chicago, IL, USA) software package. One-way ANOVA was used to compare means of the data obtained from physiochemical and SAT analyses. Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means at a 5% significance level.

4.3. Results and Discussion

4.3.1. Proximate composition, salt, WHC and cook loss

Protein (13.14–15.60%), moisture (52.81–55.71%) and fat (18.79–20.02%) contents of fresh pork sausages were not influenced by seaweed type or addition level (Table 4.2). Seaweeds employed in this study contained protein (5.57–32.03%), moisture (8.99–16.31%) and fat (1.02–2.03%) contents but at the inclusion levels employed in this study (1%, 2.5% and 5%), did not significantly impact upon sausage composition (Chapter 3, section 3.3.1). Kim *et al*. (2010) reported similar results in breakfast sausages with added sea tangle at increasing inclusion levels and decreasing pork meat content.

However, the ash content of pork sausages containing PP, SS and IW at 2.5% and 5% and nori at 5%, were higher ($P<0.05$) than the control sample. Pork sausages containing brown seaweeds SS and IW had significantly ($P<0.05$) higher ash content

and these levels increased further with increasing seaweed addition. This observation corresponds with published results observed for sea tangle, a brown seaweed which too had been incorporated into breakfast sausages Kim *et al*. (2010). From our previous study on these seaweeds, brown seaweeds have higher ash content than the red seaweeds, hence the increasing ash content with increasing brown seaweed addition (Chapter 3, section 3.3.1). Additionally, pork sausages containing red seaweeds PP and nori at 5% had higher ($P<0.05$) ash content than those containing 1% and 2.5% (Table 4.2). Results from Chapter 3, section 3.3.1 reported the ash content of these red seaweeds to range from 17.2–25.6% and thus showed no significant difference in either ash content between sausages formulated to contain 1% and 2.5% red seaweeds (Table 4.2). Sausage samples SS5%, IW5% and PP5% had higher ($P<0.05$) salt contents than the control products; however, sausage samples containing nori at 5% did not significantly differ to the control. The salt content of these seaweed-containing sausages was contributed directly by the addition of seaweeds, as had previously been quantified (Chapter 3, section 3.3.1).

In comparison to the control, sausages containing SS and IW at 2.5 and 5%, and nori at 5% displayed higher ($P<0.05$) WHC and PP at 1% had a lower ($P<0.05$) WHC. No significant difference was observed within SS and IW seaweed sausage treatment (Table 4.2). However, sausages containing PP and nori at 5% had higher ($P<0.05$) WHC than sausages containing these seaweeds at 1%. Sausages formulated with PP held less water than other seaweed sausage treatments at the different inclusion levels. Results from Chapter 3, section 3.3.7 also reported the WHC of SS, IW and nori to be greater than that of PP. Cook loss was unaffected ($P>0.05$) by the addition of

seaweeds into sausage formulations when compared to the control and within each seaweed (Table 4.2). Sausage samples significantly lost the same amount of moisture and fat during cooking as samples contained similar water and fat contents (Table 4.1) and underwent identical cooking process.

Table 4.2. Proximate composition, salt, WHC and cook loss (%) of control sausage and sausages containing 1%, 2.5% and 5% brown and red seaweed species.

Treatment		Protein	Moisture	Fat	Ash	Salt	WHC	Cook loss
Control		14.09±0.47^{a}	54.46±0.89^{abc}	20.02±0.58^{a}	2.23±0.04^{a}	1.47±0.10^{a}	63.73±1.62^{ab}	29.45±0.79^{abc}
SS	1%	13.14±0.29^{a}	55.71±0.75^{c}	19.65±0.19^{a}	2.40±0.07^{a}	1.61±0.03^{a}	69.25±1.96^{bdef}	30.27±1.72^{abc}
	2.5%	13.72±0.51^{a}	55.63±0.22^{bc}	19.14±0.53^{a}	2.85±0.05^{bc}	1.77±0.16^{abc}	70.96±3.48^{def}	28.57±0.80^{abc}
	5%	14.31±1.21^{a}	54.02±1.65^{abc}	18.79±0.33^{a}	3.39±0.23^{de}	2.10±0.01^{bcd}	74.95±1.36^{f}	25.05±2.37^{a}
IW	1%	13.61±0.17^{a}	55.03±0.41^{abc}	19.55±0.29^{a}	2.50±0.02^{ab}	1.61±0.05^{a}	67.47±1.50^{bde}	31.98±2.01^{bc}
	2.5%	13.48±0.36^{a}	54.32±0.71^{abc}	19.29±0.42^{a}	3.02±0.10^{cd}	1.86±0.13^{abcd}	71.18±0.48^{def}	30.52±5.48^{abc}
	5%	13.84±0.63^{a}	54.89±1.00^{abc}	18.99±0.42^{a}	3.66±0.02^{e}	2.26±0.19^{d}	73.20±2.58^{ef}	29.97±2.49^{abc}
PP	1%	13.38±0.13^{a}	53.82±1.34^{abc}	19.69±0.70^{a}	2.48±0.04^{ab}	1.60±0.29^{a}	55.05±1.92^{c}	33.23±0.45^{c}
	2.5%	14.38±0.62^{a}	53.52±0.64^{abc}	19.25±0.20^{a}	2.90±0.06^{bc}	1.80±0.19^{abc}	60.66±0.46^{ac}	31.04±0.72^{abc}
	5%	14.51±1.01^{a}	53.13±0.42^{ab}	18.79±0.22^{a}	3.67±0.44^{e}	2.21±0.10^{cd}	64.98±2.43^{ab}	27.38±0.69^{abc}
Nori	1%	14.04±0.34^{a}	53.43±0.29^{abc}	19.88±0.20^{a}	2.38±0.04^{a}	1.47±0.08^{a}	66.14±2.66^{abd}	28.29±2.25^{abc}
	2.5%	14.43±0.36^{a}	52.81±0.74^{a}	19.45±0.69^{a}	2.54±0.13^{ab}	1.62±0.19^{a}	69.30±1.04^{bdef}	26.14±0.73^{ab}
	5%	15.60±0.88^{a}	53.09±0.86^{ab}	18.90±0.12^{a}	3.17±0.10^{cd}	1.73±0.17^{ab}	73.36±2.06^{ef}	25.76±1.74^{ab}

^{abcdef} % Mean values (± standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

4.3.2. Surface colour of pork sausages

The surface lightness (L*) values of fresh sausages containing PP and IW at 2.5% and 5%, nori at 1%, 2.5% and 5% and SS at 5% were lower ($P<0.05$) than those observed for control sausage samples. Kim *et al*. (2010) reported comparable results, in which seaweed sausages had decreased lightness (L*) in comparison to the control due to the dark colour of seaweeds. Surface redness (a*) values decreased ($P<0.05$) in all seaweed-containing sausage samples, relative to the control, except for sausages containing nori at 1%. The addition of IW at levels of 2.5% and 5% increased the greenness (-a* values) of sausages. Kim *et al*. (2010) also reported negative a* values for sausages containing higher brown seaweed content (3% and 4%). This may be due to the higher contents of carotenoids such as fucoxanthin and chlorophyll c in some brown seaweeds that are responsible for their green colour (Shannon and Abu-Ghannam, 2017; Stengel *et al*., 2011). Similar to the lightness (L*), the yellowness (b*) values decreased ($P<0.05$) in sausages containing PP and IW at levels of 2.5% and 5%, and nori at 1%, 2.5% and 5%.

Also, within each seaweed species treatment, sausages containing SS at 5% had significantly lower a* values compared to samples containing SS at 1%. The L* value of IW pork sausages decreased ($P<0.05$) with increasing seaweed addition, 1% IW sausages had higher ($P<0.05$) a* values than the other seaweed concentrations, and higher ($P<0.05$) b* value than the 5% IW-containing sausages. Pork sausages containing PP at 1% had higher ($P<0.05$) L* and b* values than the 5% treatment. Sausages containing 5% nori had lower ($P<0.05$) L* values than the other inclusion levels and 1% nori sausages had higher ($P<0.05$) a* and b* values than the 5% nori-

containing sausages. Although not statistically significant, with increasing seaweed addition, L*, a* and b* values decreased (Table 4.3). Colour changes in pork sausages were attributed to various pigments present in brown and red seaweeds such as phycobiliproteins, carotenoids and chlorophyll (Kraan, 2013).

Table 4.3. CIE L*a*b* values of control sausage and sausages containing 1%, 2.5% and 5% brown and red seaweed species.

Treatment		L*	a*	b*
Control		70.45±0.25^{a}	6.25±0.16^{a}	13.87±0.29^{a}
SS	1%	66.54±3.33^{abc}	4.29±1.08^{bc}	13.35±0.53^{ab}
	2.5%	64.90±1.96^{abcd}	2.97±0.15^{cde}	13.16±0.30^{ab}
	5%	61.73±3.39^{bcde}	1.42±1.05^{e}	12.77±1.12^{abc}
IW	1%	66.15±0.90^{abc}	2.05±0.96^{de}	12.39±0.71^{abc}
	2.5%	59.71±2.22^{de}	-0.65±0.26^{f}	10.90±0.71^{bcd}
	5%	53.29±2.44^{fg}	-2.01±0.20^{f}	9.55±0.93^{de}
PP	1%	67.78±2.64^{ab}	3.94±0.58^{bcd}	11.62±0.94^{abcd}
	2.5%	62.21±0.47^{bcd}	3.04±0.46^{cde}	9.44±0.65^{de}
	5%	59.13±1.64^{def}	2.54±1.00^{cde}	8.03±1.54^{ef}
Nori	1%	61.09±1.71^{cde}	5.29±0.37^{ab}	10.34±1.53^{cde}
	2.5%	56.05±1.72^{ef}	4.19±0.59^{bc}	8.22±0.63^{ef}
	5%	48.96±2.02^{g}	2.70±0.33^{cde}	6.19±0.70^{f}

^{abcdef} % Mean values (± standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

4.3.3. Texture profile analysis (TPA) of cooked pork sausages

The texture profile of cooked pork sausages containing brown and red seaweeds is shown in Table 4.4. In comparison to the control, texture profile analysis (TPA) results indicated that hardness increased ($P<0.05$) in sausages containing nori and IW at 5%. Springiness values for sausages containing PP at 2.5% and 5% decreased ($P<0.05$) compared to the control. No significant differences were observed in relation to seaweed-sausages' cohesiveness values in comparison to the control. The addition of nori seaweed at 5% increased ($P<0.05$) the chewiness of sausage samples when compared to the control.

Within each seaweed species treatment, sausages incorporated with 1% PP were harder ($P<0.05$) than that of 5% PP, and sausages containing 5% nori and IW were harder ($P<0.05$) than sausages containing 1% and 2.5% of these seaweeds. Although not statistically significant, with increasing addition of PP resulted in the reduction of hardness, springiness and chewiness values in sausage samples. However, the incorporation of SS, IW and nori increased the values of hardness and chewiness. Kim *et al*. (2010) reported higher hardness values in sausages with increasing sea tangle included and demonstrated no effect on product cohesiveness.

Brown and red seaweed species contain dietary fibres such as galactans, agar, carrageenan, alginate, fucans and laminarans, all of which have gelling and thickening properties and can influence the textural properties of pork sausages (Brownlee *et al*., 2012; Thebaudin *et al*., 1997). The type and quantity of these dietary fibres present in these seaweeds may be responsible for the reduction in TPA (hardness, springiness and chewiness) values with increasing PP addition and an increment in

TPA (hardness, springiness and chewiness) with increasing SS, IW and nori addition. The addition of seaweeds into meat products such as patties and sausages have also been reported to increase hardness values (Jeon and Choi, 2012; Kim et al., 2010).

Table 4.4. TPA of control sausage and sausages containing 1%, 2.5% and 5% brown and red seaweed species.

Treatment		Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N*mm)
Control		42.81±3.47^{ab}	0.862±0.02^{abc}	0.664±0.04^{abc}	24.41±2.05^{abc}
SS	1%	35.67±1.83^{ab}	0.893±0.03^{ab}	0.686±0.01^{ab}	21.80±1.09^{ab}
	2.5%	45.23±6.11^{ab}	0.903±0.00^{a}	0.669±0.01^{abc}	27.32±3.94^{bc}
	5%	46.62±9.07^{ab}	0.843±0.04^{abcd}	0.704±0.02^{a}	27.76±6.34^{bc}
IW	1%	41.49±1.62^{ab}	0.891±0.02^{ab}	0.671±0.01^{abc}	24.89±1.26^{abc}
	2.5%	44.95±3.36^{ab}	0.852±0.01^{abc}	0.664±0.03^{abc}	25.38±3.05^{abc}
	5%	64.13±3.62^{cd}	0.813±0.04^{bcd}	0.620±0.03^{c}	32.13±3.24^{cd}
PP	1%	50.07±6.32^{bc}	0.819±0.03^{abcd}	0.618±0.01^{c}	25.11±2.59^{abc}
	2.5%	41.18±4.76^{ab}	0.783±0.03^{cd}	0.639±0.03^{bc}	20.58±2.07^{ab}
	5%	34.20±2.21^{a}	0.759±0.03^{d}	0.650±0.02^{abc}	16.91±0.78^{a}
Nori	1%	46.20±2.18^{ab}	0.871±0.02^{ab}	0.672±0.02^{abc}	27.10±2.14^{bc}
	2.5%	48.29±5.74^{ab}	0.825±0.05^{abcd}	0.682±0.01^{abc}	27.20±3.62^{bc}
	5%	68.60±6.61^{d}	0.880±0.02^{ab}	0.623±0.01^{bc}	37.42±2.82^{d}

^{abcd} % Mean values (± standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

4.3.4. Sensory analysis of cooked pork sausages

The sensory results for control and seaweed-containing sausages are shown in table 4.5. In comparison to the control, sensory analysis indicated that inclusion of nori and IW at 5% negatively influenced the liking of cooked pork sausage appearance. Sausage aroma scores also decreased ($P<0.05$) in cooked sausages containing PP, SS and IW at a 5% inclusion level. Liking of texture was not significantly affected by the addition of seaweed species. Addition of PP at 2.5% and 5%, SS and IW at 5% decreased ($P<0.05$) flavour scores for cooked pork sausages. Overall sensory acceptability decreased ($P<0.05$) in cooked sausages containing PP at 2.5% and 5%, and SS and IW at 5%. From instrumental colour analysis, brown and red seaweeds influenced the colour of pork sausages. From previous studies, the liking and perception of seaweed-containing meat product appearance (colour) by consumers were reduced in comparison to the control (Chun *et al*., 1999; Kim *et al*., 2010). However, consumers liking of appearance of seaweed-containing sausages in this study were comparable to that of the control. Additionally, food product appearance (colour) is not as relevant to consumers who purchase products based on a perceived healthiness associated with it (Kwon *et al*., 2003). The observed trends suggest that the participants in this study exhibited a level of familiarity with the intake of seaweed, which consequently influenced their positive inclination towards these products. Consumers (n=25) preferences for sausage treatments are shown in figure 4.1 and they followed the order: control > nori 1% ≥ SS1% > nori 2.5% > IW 1% > SS 2.5% > SS 5% ≥ nori 5% ≥ IW 5% > PP 1% ≥ PP 2.5% > PP 5% = IW 2.5%.

Table 4.5. Sensory (hedonic) scores of control sausage and sausages containing 1%, 2.5% and 5% brown and red seaweed species.

Treatment		Appearance	Aroma	Texture	Flavour	Overall Acceptability
Control		6.78±1.76^{ab}	6.87±1.46^{ab}	5.72±2.07^{ab}	6.70±1.69^{ab}	6.78±1.76^{ab}
SS	1%	7.18±1.20^{a}	6.89±1.48^{ab}	6.53±1.46^{a}	7.39±1.59^{a}	7.27±1.45^{a}
	2.5%	6.63±1.02^{ab}	5.23±2.03^{bcd}	5.71±1.51^{ab}	5.04±2.09^{bcd}	5.23±1.71^{bcde}
	5%	5.78±2.03^{abc}	4.12±2.41^{d}	5.07±2.01^{ab}	3.14±2.69^{d}	3.81±2.50^{e}
IW	1%	6.37±1.13^{ab}	6.82±1.22^{ab}	5.76±1.96^{ab}	6.68±1.28^{ab}	6.26±1.30^{abc}
	2.5%	5.24±1.87^{bc}	5.38±1.85^{bcd}	5.65±1.96^{ab}	5.32±1.84^{abc}	5.55±1.63^{abcde}
	5%	3.19±1.99^{d}	4.42±1.97^{cd}	4.37±1.58^{b}	4.38±2.41^{cd}	4.12±2.08^{de}
PP	1%	6.96±1.65^{ab}	7.23±1.16^{a}	5.84±1.98^{ab}	5.83±2.08^{abc}	5.91±1.77^{abcd}
	2.5%	6.03±1.82^{abc}	6.05±1.67^{abc}	5.29±2.20^{ab}	4.34±2.61^{cd}	4.78±2.79^{cde}
	5%	5.44±1.37^{abc}	4.79±2.25^{cd}	5.01±2.05^{ab}	3.23±2.28^{d}	4.17±2.14^{de}
Nori	1%	7.01±1.36^{a}	6.87±1.40^{ab}	5.71±1.63^{ab}	7.08±1.57^{ab}	6.98±1.60^{ab}
	2.5%	6.23±2.00^{abc}	6.87±1.23^{ab}	5.35±2.25^{ab}	6.82±1.63^{ab}	6.23±1.71^{abc}
	5%	4.60±2.22^{cd}	6.02±1.17^{abc}	5.44±1.73^{ab}	6.58±1.99^{ab}	6.18±1.84^{abc}

^{abcde} Mean values (± standard deviation) in the same column bearing different superscripts are significantly different ($P<0.05$).

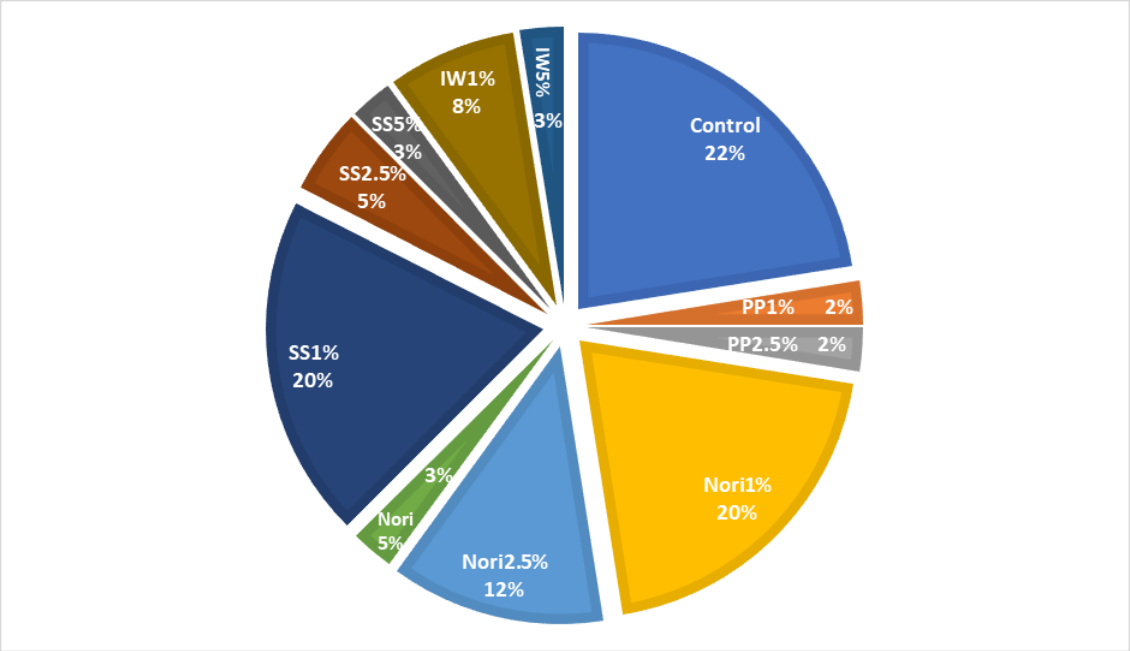


Figure 4.1. Pie chart representing consumer (n= 25) preference.

4.4. Conclusions

The incorporation of edible and commercially available brown and red seaweed species did not exert significant effects on the protein, moisture and contents of fresh pork sausages. However, the ash content of seaweed sausages increased compared to the control. Seaweed addition had a notable impact on water holding capacity (WHC), and cook loss was unaffected by seaweed addition. Moreover, addition of seaweeds (nori, SS and IW) at a 5% inclusion level and PP at 2.5% and 5% negatively influenced the colour, instrumental textural properties and sensory attributes of pork sausages. Colour changes in seaweed sausages were dependent on the type and quantity of pigments present in seaweeds and the inclusion level of seaweeds used. Regardless of the impact of these seaweeds on the colour of the sausage samples, panellists' liking of appearance was comparable to that of the control. Sensory results indicated that consumers preferred sausages with lower seaweed content and liking of seaweed sausages were in the order nori > SS > IW > PP. This study demonstrated that, in pork sausages, a maximum acceptable inclusion level for PP is 1% and 2.5% for the other seaweed species examined without negatively influencing the quality, functionality and sensory acceptance. Utilising these seaweed species at the acceptable inclusion levels determined in the present study may enhance the healthiness, such as sodium, salt and fat reduction, in pork sausages to meet consumer preferences and market demands for healthier and more sustainable food options. Further research is warranted to explore the potential of seaweed-based ingredients in enhancing the nutritional profile and sensory appeal of meat products while maintaining product quality and consumer acceptance.

Chapter 5.

Utilisation of brown Irish seaweed species (sea spaghetti- *Himanthalia elongata* and Irish wakame- *Alaria esculenta*) as partial fat and salt replacers in pork sausages: physicochemical, sensorial and shelf-life characteristics

Abstract

Edible brown seaweeds, sea spaghetti (SS) and Irish wakame (IW) were incorporated at 2.5% into reduced fat (20%, 15% and 10%) and salt (1.5%, 1% and 0.5%) pork sausages. The physicochemical and sensory characteristics of reformulated sausages were analysed. Subsequently, shelf-life evaluation (lipid oxidation and microbiological analysis) was performed on selected formulations stored under aerobic (AP), MAP70/30 (70% N_2 :30% CO_2), MAP80/20 (80% O_2 :20% CO_2) and vacuum (VP) conditions. Relative to the control, seaweed sausages containing 10% fat had lower ($P<0.05$) protein and 1.5% salt seaweed sausages had higher ($P<0.05$) ash content. The addition of seaweed did not affect the pH of reduced fat and salt sausages and cook loss increased in reduced fat sausages. Reduced fat and salt seaweed sausages were darker than the experimental controls. Based on sensory results, the selected sausage formulations using SS and IW were 10% fat, 0.5% salt (SS10f/0.5s) and 15% fat, 1% salt (IW15f/1s), respectively. The SS treatments were generally well-received by the panellists, but the IW treatments had relatively lower sensory scores. With regards to shelf life of selected seaweed sausage formulations, MAP70/30 (70% N_2, 30% CO_2) and VP (Vacuum packaging) were the most effective for lipid oxidation and TVC (total viable counts), respectively.

5.1. Introduction

Pork meat accounts for approximately 40% of global meat consumption. The palatability, nutritional quality (protein, vitamins and minerals) and versatility of pork meat render it suitable for the manufacture of a range of processed pork products such as cured hams, puddings and sausage (fresh, emulsion or fermented/dried) type products (Keenan, 2016). Fresh pork sausages are comminuted type processed meat products where typically, minced or ground pork meat, (back) fat and a range of ingredients (water, cereal (rusk) or protein substitutes) and additives (salt, phosphates, seasoning, spices and preservatives) are mixed (chopped) together and filled into edible collagen casings. Currently, on the Irish market, fresh pork sausages typically contain 24-28% fat and 1.7-2.4% salt. Pork back fat, utilised in sausage formulations, contains saturated fat at levels of up to 40% (Asuming-Bediako *et al*., 2014).

Consumption of processed meats and ingredients contained therein (fat and salt (sodium)) are often associated with having a negative impact on human health, leading to various conditions such as increased blood pressure, obesity, cardiovascular disease and cancer. As a consequence, the Department of Health and the Food Reformulation Taskforce in Ireland aims to reduce the intake of calories, saturated fat, sugar and salt in the Irish diet. By 2025, a 10% reduction in saturated fat and salt intake is targeted (FSAI, 2022). Such directives have prompted research into fat and salt reduction strategies for processed pork products, including pork sausages.

Fat and salt, as ingredients in processed meat formulations, play an important role with respect to product quality and shelf-life. Fat acts as a reservoir for flavour compounds and contributes positively to product texture (Naga Mallika *et al*., 2009) and consumer acceptability. Salt addition enhances product flavour, improves preservation by reducing water activity (Taormina, 2010) and increases water holding capacity through the extraction of myofibrillar proteins, resulting in enhanced cohesiveness and adhesiveness properties (Durack *et al*., 2008; Inguglia *et al*., 2017). In addition to water binding, salt addition also contributes to the fat binding properties of meat products resulting in reduced cook losses and enhanced textural characteristics (Ruusunen *et al*., 2003).

Research into fat and/or salt replacement and reduction strategies in processed meats has focused on ingredients which would mimic fat and salt functionality in product formulations. Ingredients such as konjac flour, vegetable oil, starch, inulin and pectin have been examined as fat replacers in a range of sausage-type products (Asuming-Bediako *et al*., 2014; Garcia-santos *et al*., 2019; Méndez-Zamora *et al*., 2015). In addition, salt (partial or complete replacement) can be substituted with other mineral salts such as potassium chloride (KCl), magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) (Tobin *et al*., 2013). Simultaneous reduction of both fat and salt has been explored in pork products such as pork sausages (Tobin *et al*., 2013) and with replacers such as mushroom flours in frankfurters (Cerón-Guevara *et al*., 2020) and inulin in bologna sausage (de Souza Paglarini *et al*., 2021).

The unique compositional, nutritional and technological properties of seaweeds may offer potential for their use as replacer ingredients in meat products (Fernández-

Martín et al., 2009; López-López, Cofrades, and Jiménez-Colmenero, 2009). A limited number of previously reported studies have examined the use of brown seaweed species such as wakame (*Undaria pinnatifida*) and sea spaghetti (*Himanthalia elongata*) as fat and/or salt replacers in beef patties and frankfurters at inclusion levels ranging from 1 – 3.3% (Jiménez-Colmenero *et al*., 2010; López-López *et al*., 2011; Vilar *et al*., 2020). AlgySalt®, a commercial salt replacer and seaweed extract manufactured from a range of seaweed species, was examined in turkey/beef fat sausages with limited success due to increased sausage hardness (Triki *et al*., 2017). Brown seaweeds are rich in polysaccharides such as fucoidan, laminarin and alginates. These compounds can increase viscosity, provide thickening properties but may also confer textural characteristics similar to fat in processed meats. Brown seaweeds also contain a high level of minerals (e.g. potassium) and amino acids such as aspartic and glutamic acids, which may positively influence saltiness and umami flavour profiles in pork products (Mabeau *et al*., 1992). The use of commercially available Irish brown seaweed species (sea spaghetti (*Himanthalia elongata*) and Irish wakame (*Alaria esculenta*)) as partial fat and salt replacers in fresh pork sausages has not been examined and merits investigation. Since seaweeds have an intense flavour profile, a maximum acceptable inclusion level of 2.5% for sea spaghetti and Irish wakame in fresh pork sausages was demonstrated in Chapter 4.

The objective of this study was to investigate the effectiveness of Irish brown seaweed species (sea spaghetti (*Himanthalia elongata*) and Irish wakame (*Alaria esculenta*)) as partial fat (25, 20, 15 and 10%) and salt (2, 1.5, 1 and 0.5%) replacers in fresh pork sausage formulations at an inclusion level of 2.5%. Physicochemical,

sensory and the shelf-life stability (lipid oxidation and microbiological analysis) of selected pork sausage formulations stored in various packaging formats was also examined.

5.2. Materials and Methods

5.2.1. Chemicals and reagents

Kjeltabs, sodium chloride (NaCl), hydrogen peroxide (H_2O_2), boric acid (H_3BO_3), hydrochloric acid (HCl) and sulphuric acid (H_2SO_4), Trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA) were supplied by Sigma- Aldrich Ireland Ltd., Vale Road, Arklow, Wicklow, Ireland. Silver nitrate and Potassium chromate (K_2CrO_4) were purchased from Fisher Scientific, United Kingdom. Potassium dichromate ($K_2Cr_2O_7$) was supplied by BDH Limited Supplies, Poole, England. All solvents were of analytical grade.

5.2.2. Raw materials

Brown seaweed species (*Himanthalia elongata* (Sea spaghetti SS) and *Alaria esculenta* (Irish wakame IW)) were purchased from Wild Irish Seaweed, Co. Clare, Ireland. According to supplier specification, all seaweeds were hand-harvested off the coast of Co. Clare, Ireland, 100% naturally grown and sustainably harvested and organically certified. Seaweed species were air-dried, dehumified, and milled prior to purchase. The average particle size of brown seaweeds was approximately 2.5 mm (Tyuftin *et al*., 2021).

Pork oyster muscle and pork backfat were supplied by Ballyburden Meat Processors (Cork, Ireland). Food-grade salt was obtained from British salt Ltd. (Cheshire, UK). A custom formulated reduced salt sausage seasoning (0.39% salt) containing rusk [wheat flour (calcium carbonate, Iron, Niacin, Thiamin), salt], sugar, dextrose, stabiliser (E450, E452), flavour enhancer (E621), preservative (E223), antioxidant (E301), natural flavourings, anticaking agent (E551), rapeseed oil, hydrolysed vegetable protein (soya, maise)] was obtained from Redbrook Ingredient Services (Mulhuddart, Dublin 15, Ireland). Rusk [Wheat flour (wheat flour, calcium carbonate, Iron, Niacin, Thiamin), salt, raising agent E503] and collagen casing were purchased from Viscofan UK Ltd. (Sevenoaks, Kent, UK).

5.2.3. Sausage manufacture

Fresh pork sausages (4.3 kg batches) (Control (C)) were formulated containing 41.1% pork oyster muscle, 25% pork back fat (f), 18.5% water (50:50, water: ice), 11.4% rusk and 2.0% seasoning and 2% salt (s) (0.39% salt from the sausage seasoning, 0.14% from the rusk plus 1.5% salt added as an ingredient into the formulation) (Table 5.1). The control formulation, designated C25f/2s, was based on the average composition of pork sausages typically available on the Irish market. Additional batches of pork sausages (n = 9) were manufactured with a sequential reduction of fat (f) (20%, 15% and 10%) and salt (s) (1.5%, 1% and 0.5%) levels (at each fat level) resulting in 20f/1.5s, 20f/1.0s, 20f/0.5s, 15f/1.5s, 15f/1.0s, 15f/0.5s, 10f/1.5s, 10f/1.0s and 10f/0.5s treatments. Seaweed species (SS or IW) were added to the additional pork

sausage batch formulations at a level of 2.5%, previously identified as the maximum acceptable inclusion level in pork sausages (Chapter 4).

Prior to sausage manufacture, pork oyster and backfat were minced through a 4 mm perforated plate (Talsabell SA., Valencia, Spain). Pork oyster, half of the water, salt, and the seasoning was mixed for 60 seconds at high-speed using a bowl chopper (Seydleman Bowl chopper, Burgstallstraße, Germany). The backfat and various seaweeds were added and mixed at high speed for 30 seconds. The remaining water was added, and the batter was mixed for an additional 30 seconds at high speed. Finally, the rusk was added and mixed at low speed for 30 seconds. Sausage batter formulations were stuffed using a piston-type sausage filler (Mainca UK Ltd., Berkshire, UK) into 21mm diameter collagen casings and hand-linked into individual pork sausages (~ 10 cm in length).

Table 5.1. Sausage treatments and formulations (%).

Treatment	Pork oyster	Pork backfat	Water	Rusk	Seasoning	Salt	Seaweed
C25f/2s*	41.1	25	18.5	11.4	2	2.0	0
20f/1.5s	44.1	20	18.5	11.4	2	1.5	2.5
20f/1.0s	44.6	20	18.5	11.4	2	1.0	2.5
20f/0.5s	45.1	20	18.5	11.4	2	0.5	2.5
15f/1.5s	49.1	15	18.5	11.4	2	1.5	2.5
15f/1.0s	49.6	15	18.5	11.4	2	1.0	2.5
15f/0.5s	50.1	15	18.5	11.4	2	0.5	2.5
10f/1.5s	54.1	10	18.5	11.4	2	1.5	2.5
10f/1.0s	54.6	10	18.5	11.4	2	1.0	2.5
10f/0.5s	55.1	10	18.5	11.4	2	0.5	2.5

* Control

5.2.4. Physicochemical properties

5.2.4.1. Proximate composition

The protein (Kjeldahl) and ash (muffle furnace at 550 ^{o}C) contents of sausage samples were analysed following AOAC Methods 954.01 and 942.05, respectively (AOAC, 1999). The moisture and fat content of sausages were determined using a SMART Trac system (CEM GmbH, Kamp- Lintfort, Germany). Results were expressed as g/100 g of sample.

5.2.4.2. Salt analysis

The salt content of sausage samples was determined by titration using silver nitrate (Kirk and Sawyer, 1991). The silver nitrate (0.1 N $AgNO_3$) solution was standardised

against 0.1% sodium chloride (NaCl). Sausage samples were ashed at 550 ^{o}C in a muffle furnace as described in section 5.2.4.1. Ash samples were subsequently washed into conical flasks with 20 ml distilled water. The indicator (potassium chromate and potassium dichromate) (2 ml) was added and standardised silver nitrate was used to titrate the solution from a clear yellow colour to an opaque light orange. Blank titrations were performed using 20 ml distilled water. Salt content was calculated using the following equation:

$$\% \text{ salt} = \frac{\text{Titre for sample (ml)} - \text{Titre for blank (ml)}}{\text{Mass of sample (g)}} \times \text{Molarity of } AgNO_3 \times 5.844$$

5.2.4.3. pH determination

Fresh sausage samples (5 g) were homogenised for 1 min at 24,000 rpm in 45 ml distilled water using an Ultra Turax T25 homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen, Germany). The pH was measured at 20 ^{o}C using a pH meter (Seven Easy portable, Mettler-Toledo GmbH, Schweizenbach, Switzerland).

5.2.4.4. Instrumental colour analysis

The colour of fresh sausage samples was measured according to the CIE L*a*b* colour system. The samples were allowed to equilibrate at room temperature for 30 minutes before measuring. The surface colour was measured using a Minolta chromameter (CR400, Minolta Camera Co.Ltd, Osaka, Japan) with an 11mm-

diameter aperture and D65 illuminant. The chromameter was calibrated using a white tile (L=97.79, a=-0.11, b= 2.69).

5.2.4.5. Texture profile analysis

The texture profile analysis (TPA) of sausage samples was carried out using a texture analyser TA-XF10S1.5i (Stable Micro System, Surrey, England) based on a method described by Bourne (1978). Cooked sausage samples were allowed to equilibrate at room temperature for 30 minutes before measuring. The samples were cut into cylindrical slices (10 mm thickness), and four pieces were measured per sausage sample. The samples were subjected to a two-cycle compression using a 30 kg load cell. All samples were compressed to 40% of their original height using a cylindrical probe (SMP/35 compression plate) at a cross-head speed of 1.5 mm/s. Determining factors include; hardness (N), the maximum force required for the first compression; springiness (mm), distance the sample recovers after initial compression; cohesiveness (dimensionless), the ratio of positive force area during the second compression and chewiness (N*mm), the product of gumminess and springiness.

5.2.4.6. Water Holding Capacity

The water holding capacity (WHC) was measured using the method described by Lianji and Chen (1989). Fresh sausage samples (10 g) were weighed into glass jars and heated in a water bath for 10 min at 90 ^{o}C. After heating, the samples were removed, wrapped in cheesecloth, and placed in 30 ml centrifuge tubes lined with cotton wool at the bottom of each tube. The samples were centrifuged (Avanti J-E centrifuge,

Beckman Coulter, California, USA) at 13,300 g for 10 min at 4 °C. After centrifugation, the cheesecloth was removed, and samples were reweighed. WHC was calculated using the following calculation:

$$\% \text{ Water holding capacity} = 1 - \frac{(B-A)}{(B \times M)} \times 100$$

B is the sample's weight before heating, A is the weight of the sample after heating and centrifuging, and M is the % moisture of the sample (section 5.2.4.1).

5.2.4.7. Cook loss

Sausage weights were recorded before and after cooking. Sausages were cooked in a Zanussi oven at 180 °C for 22 min until an internal temperature of 72 °C was reached. Cook loss was calculated using the following equation:

$$\% \text{ Cook loss} = \frac{\text{Raw weight-cooked weight}}{\text{Raw weight}} \times 100$$

5.2.5. Sensory Analysis (Sensory acceptance testing)

Sensory acceptance testing (SAT), also known as affective testing or hedonic testing, is a sensory technique used to assess product acceptability and liking/preference or its unique sensory properties (Cardello and Jaeger, 2010).

Sausages were cooked as described in section 5.2.4.7, cooled and cut into cylindrical pieces (~2 cm). sausage samples were placed on plates and randomly assigned three digit codes. Sensory acceptance testing (hedonic (liking)) was carried out using an untrained consumer panel (n=25) (different set of panellists from chapter 4), who were familiar with the taste of pork sausages (Stone *et al*., 2012). Sensory analysis was conducted in sensory booths conforming to international standards (ISO, 2014)

at room temperature. The assessors were presented 20 sausages samples (10 treatments x 2 replicates). This was carried out over two sessions, with the same set of panellists (n=25), to reduce panellist fatigue. Water for palate cleansing between samples was also provided. Assessors were asked to indicate their scores in relation to the following hedonic terms; appearance, aroma, flavour, texture, and overall acceptability on a 10 cm hedonic scale (1= extremely dislike and 10=extremely like). All subjects gave informed consent for inclusion before participating in the sensory study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the University College Cork Social Research and Ethics Committee (Log 2021-188).

5.2.6. Packaging and shelf-life of pork sausages

Optimum packaging conditions for the control (C25f/2s) and two selected sausage formulations (SS10f/0.5s and IW15f/1s) was investigated using four packaging treatments. Sausage samples were placed in low oxygen permeable (< 1cm^3/m^2/24 h at STP) polystyrene/ethyl vinyl alcohol/polyethylene trays and over-wrapped with oxygen-permeable film (cling film) for aerobic packaging (AP) conditions. Using modified atmosphere packaging (MAP) technology, trays containing the sausage treatments were flushed with 80% O_2: 20% CO_2 (MAP80/20) or 70% N_2: 30% CO_2 (MAP70/30) 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) (Salami *et al*., 2020).

Sausage samples were also placed in vacuum pouches (polyamide/ polyethylene 90, O_2: 50 cm^3 /m^2 /24 h STP, N_2: 10 cm^3 /m^2 /24 h STP, CO_2: M150 cm^3 /m^2 /24 h STP) and vacuum packaged (VP) using a vacuum machine (Henkelmann, Polar 80, Henkelmann, Titaniumlaan 10, 5221 CK's-Hertogenbosch, Netherlands). All packaging treatments (AP, MAP80/20, MAP70/30 and VP) were stored for up to 27 days at 4 ^oC. Shelf-life measurements included lipid oxidation and microbiological analysis.

5.2.6.1. Lipid oxidation

Lipid oxidation measurements were carried out following the method described by (Siu and Draper, 1978) on aerobically packaged (AP), MAP80/20, MAP70/30 and vacuum packaged (VP) sausages on days 1, 6, 13, 20 and 27 stored at 4 ^oC. Results were expressed as TBARS in mg malondialdehyde (MDA)/kg sausage.

5.2.6.2. Microbiological analysis

Microbiological analyses (Total viable counts (TVC), *Escherichia coli (E. coli)* and *Salmonella*) were determined as described by (O' Neill *et al*., 2018).

Raw sausage samples (10 g) were weighed aseptically into a stomacher bag and a primary 10-fold dilution was carried out by adding 90 ml of sterile maximum recovery diluent (MRD) (Oxoid, Basingstoke, U.K.), stomaching (Steward Stomacher 400 Lab Blender, London, UK) the samples for 3 minutes, and then serially diluting the homogenates by 10-fold using MRD. In order to count TVC, 1 ml of each suitable dilution in the middle of compact dry-total count plates (20 cm^2) (Nissui

Pharmaceutical, Co. Ltd., Japan) and incubated for 48 hours at 37 °C. *E. Coli* was detected using Compact Dry EC plates (Nissui Pharmaceutical, Japan). 1 ml of each suitable dilution was inoculated in the middle of the plates and incubated at 37 °C for 24 hours.

Sausage samples were examined for the presence or absence of Salmonella in Compact dry SL plates at the start and finish of storage (Nissui Pharmaceutical, Co. Ltd., Japan). The pre-enrichment procedure involved weighing 25 g of the sample into a filter stomacher bag, adding 225 ml of Buffered Peptone water (Oxoid), homogenising the mixture with a stomacher for one minute, and then incubating the mixture for 24 hours at 37 °C. After removing the bag from the incubator, 0.1 ml of the enriched specimen was carefully dropped onto the sheet, approximately 1 cm from the plate's edge and 1 ml of sterile water was added adjacent to the specimen. The plates were incubated for 48 hours at 42 °C.

Analyses were carried out on AP, MAP80/20, MAP70/30 and VP sausages on days 1, 6, 13, 20 and 27 stored at 4 °C. Except for *Salmonella*, the results were given as $\log_{10}$ colony-forming units per gram of sausage (CFU/g).

5.2.7. Statistical analysis

Statistical analysis was carried out using the IBM SPSS 28.0 for Windows (SPSS, Chicago, IL, USA) software package. All analyses were performed in duplicate and three independent experimental trials were carried out. The physiochemical data of seaweed sausages was analysed using a two-way ANOVA to investigate the effects of salt level, fat level and fat x salt interactions. Tukey's post-hoc test was used to

adjust for multiple comparisons between treatment means when significance was detected at $P<0.05$.

A mixed model ANOVA was used to analyse the sensory analysis data with treatment as a fixed effect and batch and panellist as random effects. Interactions between fixed and random effects were measured and Tukey's post-hoc test was used to adjust for multiple comparisons between treatment means.

In the packaging study (lipid oxidation and microbiological analysis), a full repeated measures ANOVA was conducted to investigate the effect of packaged sausage treatments and storage time on lipid oxidation and microbial growth. Sausage treatment represented the 'between-subjects' factor and the effect of time was measured using the 'within-subjects' factor. Tukey's post-hoc test was used to adjust for multiple comparisons between treatment means when significance was detected at $P<0.05$.

5.3. Results and Discussion

5.3.1. Physicochemical properties of sausage formulations

5.3.1.1. Proximate composition

The proximate composition of sausages containing sea spaghetti (SS) and Irish wakame (IW) is shown in Table 5.2. The overall protein content of SS sausages was significantly influenced by the fat level while IW sausages were significantly impacted by the fat and salt levels. Sausage treatments SS and IW, containing 10% fat had higher ($P<0.05$) protein content relative to the control. In order to reduce fat and salt levels in sausage formulations, increasing levels of pork oyster muscle was added (Table 5.1) which may account for the variances in the protein content of sausage treatments.

The overall fat and salt levels had significant effects on the moisture content of SS and IW sausages. Sea spaghetti treatments, 20f/0.5s, 15f/0.5s, 10f/1.5s, 10f/1.0s and 10f/0.5 had higher ($P<0.05$) moisture content than the control, while all IW treatments, except 20f/1.5s, had significantly higher moisture content than the control. For a given fat level, within each SS and IW treatment, there were no significant differences in moisture content; for example, 20f/1.5s, 20f/1.0s, and 20f/0.5s were not significantly different from one another ($P>0.05$). The moisture content of SS and IW sausages also increased ($P<0.05$) with reduced fat addition. Previous studies reported the moisture contents of reduced fat frankfurter and sausages to be inversely proportional to the fat content, i.e. higher moisture in lower fat products (Cengiz and Gokoglu, 2007; Papadima and Bloukas, 1999).

The overall fat content of SS and IW incorporated sausages was significantly influenced by the fat levels. The fat content of seaweed incorporated sausages was lower ($P<0.05$) than the control (Table 5.2). Results from chapter 3, section 3.3.1 reported that seaweeds used in this study have low fat contents (1.2-2%) and at an inclusion level of 2.5%, contributed minimal fat to the various sausage formulations. López-López *et al*. (2010) also reported that the fat content of beef patties was not influenced to any significant degree owing to the low fat content of wakame (0.88%). The overall ash content of SS and IW sausages were significantly influenced by the salt level. Sea spaghetti and Irish wakame sausage treatments containing 1.5% salt contained significantly higher ($P<0.05$) ash than the control. This was attributed to the salt content (12-15%) and mineral contents of both seaweed species (Chapter 3, sections 3.3.1 and 3.3.4). Sea spaghetti sausage treatments with 1.0% and 0.5% salt and IW sausage treatments with 0.5% salt had significantly lower ash content than the control (Table 5.3), presumably due to the lower salt levels included in the formulations (Table 5.1). López-López *et al*. (2009) and Kim *et al*. (2010) also found that seaweeds affected the ash content of frankfurters and sausages, respectively.

Table 5.2. Proximate composition of reduced fat and salt pork sausages containing brown seaweeds.

Treatment	Sea spaghetti (SS)				Irish wakame (IW)			
	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Moisture (%)	Fat (%)	Ash (%)
C25f/2s	12.65±0.33^{a}	53.58±0.34^{a}	20.31±0.51^{a}	2.80±0.03^{a}	12.61 ±0.41^{a}	52.78±0.74^{a}	19.50±0.31^{a}	2.61±0.01^{a}
20f/1.5s	13.83±0.91^{abc}	54.78±1.74^{ab}	16.43±2.61^{b}	3.11±0.02^{b}	12.63 ±0.43^{a}	54.43±1.16^{ab}	16.16±0.60^{b}	3.00±0.08^{b}
20f/1.0s	13.44±0.69^{ab}	54.92±0.31^{ab}	16.56±0.44^{b}	2.45±0.07^{c}	13.05±0.25^{ab}	55.58±0.55^{b}	15.83±0.56^{b}	2.51±0.03^{a}
20f/0.5s	13.21±0.12^{ab}	56.26±0.42^{b}	15.62±0.39^{b}	2.02±0.08^{d}	12.94±0.29^{ab}	55.58±0.41^{b}	16.28±0.25^{b}	2.16±0.07^{c}
15f/1.5s	14.05±0.68^{abc}	55.64±1.37^{ab}	14.42±1.16^{b}	3.08±0.07^{b}	13.35±0.41^{abc}	58.04±0.61^{c}	12.30±0.48^{c}	3.00±0.04^{b}
15f/1.0s	13.12±0.50^{ab}	55.70±0.97^{ab}	14.40±1.47^{b}	2.50±0.04^{c}	14.00±0.61^{abc}	58.01±0.37^{c}	12.82±0.40^{c}	2.52±0.08^{a}
15f/0.5s	13.95±0.5^{abc}	57.12±0.79^{bc}	14.54±1.40^{b}	2.03±0.06^{d}	14.04±0.10^{abc}	57.72±0.27^{c}	12.65±0.38^{c}	2.03±0.07^{c}
10f/1.5s	14.67±0.46^{bc}	60.30±0.65^{d}	9.31±0.03^{c}	3.03±0.03^{b}	14.19±0.46^{bc}	59.11±0.88^{cd}	10.13±0.74^{d}	3.04±0.05^{b}
10f/1.0s	15.35±0.27^{c}	59.44±0.66^{cd}	10.31±1.41^{c}	2.57±0.03^{c}	15.77±0.97^{d}	60.21±0.63^{d}	9.11±0.25^{d}	2.64±0.03^{a}
10f/0.5s	14.60±0.43^{bc}	61.35±0.84^{d}	9.07±0.51^{c}	2.16±0.15^{d}	14.81±0.73^{cd}	60.81±0.33^{d}	9.41±0.33^{d}	2.10±0.17^{c}
Overall effects (P values) of variables (fat, salt and fat x salt interaction) on seaweed sausages' proximate composition.								
Fat	<0.001***	<0.001***	<0.001***	0.137^{ns}	<0.001***	<0.001***	<0.001***	0.131^{ns}
Salt	0.581^{ns}	0.006**	0.539^{ns}	<0.001***	0.008**	0.023*	0.463^{ns}	<0.001***
Fat * salt	0.100^{ns}	0.904^{ns}	0.863^{ns}	0.180^{ns}	0.320^{ns}	0.120^{ns}	0.098^{ns}	0.309^{ns}

^{abcd} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

P values- ns: not significant, *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

5.3.1.2. pH, salt, water holding capacity (WHC) and cook loss

The pH of SS and IW seaweeds was 5.55 and 6.29, respectively (Chapter 3, section 3.3.1). The pH values of SS and IW sausage treatments ranged from 5.71 to 5.93 and 5.82 to 5.89, respectively (Table 5.3). The overall pH of SS and IW sausages was significantly influenced by the fat and salt levels, and a fat x salt interaction was observed. In this study, the addition of seaweeds to reduced fat and salt sausages did not affect ($P>0.05$) the pH of the sausage treatments compared to the control. Although within each fat treatment, the pH value of SS sausage treatments decreased with increasing salt level. Puolanne *et al*. (2001) also observed a reduction in pH values as the amount of salt applied increased in pork sausage batter. The effect of salt on the pH of meat is most likely caused by the fact that the Cl^- ion is more strongly bound to the proteins (such as myosin) that the Na^+ ion. The adsorption of Cl^- ions with positively charged groups of myosin results in a shift of the isoelectric point; hence, lowering pH (Desmond, 2006; Hamm, 1986).

The overall salt content of SS sausage treatments was significantly influenced by the fat, salt levels and a fat x salt interaction was observed, while fat and salt levels had a significant effect on the salt content of IW sausages. Seaweed sausages (SS and IW treatments) containing 1.5% salt had salt levels comparable ($P>0.05$) to the control. The salt content of SS and IW sausages was higher than the projected levels for each formulation (0.5%, 1.0% and 1.5% salt) (Table 5.1), presumably due to the salt levels present in both brown seaweed species (12- 15%) (Chapter 3, section 3.3.1).

The overall WHC of SS sausage treatments was significantly influenced by fat level and IW samples were affected by the fat and salt levels. The WHC of SS (10f/1.5s,

10f/1.0s and 10f/0.5s) and IW (10f/1.5s and 10f/0.5s) treatments was significantly higher than the control. This increased WHC may be attributed to the increased pork meat content in sausage formulations (Table 5.1). The overall cook loss of IW sausages was significantly impacted by the fat and salt levels. The SS treatments (20f/1.5s, 10f/1.5s and 10f/0.5s) had similar ($P>0.05$) cook loss values to the control, while other treatments had higher ($P<0.05$) values. Cook loss values were higher ($P<0.05$) in all IW treatments compared to the control, except for treatment 10f/1.5s. Higher cook loss values can be attributed to the reduction of salt in seaweed formulations. Ruusunen *et al*. (2003) noted that the reduction of salt in meat products increases cooking loss due to its water- and fat- binding properties.

Table 5.3. pH, salt, water holding capacity (WHC) and cook loss of reduced fat and salt pork sausages containing brown seaweeds.

Treatment	Sea spaghetti (SS)				Irish wakame (IW)			
	pH	Salt (%)	WHC (%)	Cook loss (%)	pH	Salt (%)	WHC (%)	Cook loss (%)
C25f/2s	5.81±0.11^{abc}	1.82±0.11^{a}	63.60±1.91^{a}	22.65±1.68^{a}	5.82±0.03^{a}	1.79±0.01^{ab}	62.79±2.40^{a}	21.59±1.05^{a}
20f/1.5s	5.71±0.02^{a}	1.79±0.01^{a}	64.96±1.30^{ab}	27.04±1.19^{abc}	5.89±0.01^{a}	1.81±0.03^{a}	64.52±1.19^{a}	27.54±2.32^{c}
20f/1.0s	5.88±0.02^{bc}	1.22±0.01^{b}	64.63±1.92^{ab}	29.05±0.50^{c}	5.85±0.00^{a}	1.28±0.03^{c}	66.33±2.20^{ab}	29.72±1.13^{c}
20f/0.5s	5.91±0.01^{bc}	0.76±0.01^{c}	66.97±2.66^{abc}	29.15±1.99^{c}	5.86±0.01^{a}	0.79±0.02^{d}	65.34±3.16^{a}	29.28±1.41^{c}
15f/1.5s	5.76±0.07^{ab}	1.76±0.02^{a}	66.52±0.75^{abc}	27.62±1.19^{bc}	5.86±0.01^{a}	1.77±0.01^{ab}	67.11±1.28^{ab}	27.34±0.92^{bc}
15f/1.0s	5.88±0.02^{bc}	1.24±0.02^{b}	67.31±1.69^{abcd}	27.71±1.11^{bc}	5.86±0.00^{a}	1.25±0.03^{c}	68.13±5.50^{ab}	28.35±0.35^{c}
15f/0.5s	5.93±0.01^{c}	0.72±0.01^{c}	66.94±1.83^{abc}	29.66±1.58^{c}	5.82±0.01^{a}	0.76±0.02^{d}	65.06±7.84^{a}	31.22±1.23^{c}
10f/1.5s	5.83±0.03^{abc}	1.72±0.03^{a}	69.56±1.76^{bcd}	23.49±1.48^{ab}	5.87±0.01^{a}	1.74±0.02^{b}	73.01±1.40^{b}	23.15±2.71^{ab}
10f/1.0s	5.89±0.01^{bc}	1.21±0.02^{b}	72.21±1.74^{d}	27.81±1.17^{bc}	5.84±0.01^{a}	1.27±0.01^{c}	73.70±1.76^{b}	29.43±1.52^{c}
10f/0.5s	5.91±0.05^{bc}	0.72±0.03^{c}	70.60±1.59^{cd}	25.63±2.70^{abc}	5.82±0.01^{a}	0.74±0.02^{d}	67.97±1.90^{ab}	28.35±0.39^{c}
Overall effects (P values) of variables (fat, salt and fat x salt interaction) on seaweed sausages' pH, salt, WHC and cook loss.								
Fat	0.014*	0.003**	<0.001***	0.906^{ns}	<0.001***	0.002**	<0.001***	0.021*
Salt	<0.001***	<0.001***	0.430^{ns}	0.939^{ns}	<0.001***	<0.001***	0.046*	<0.001***
Fat x salt	0.010**	0.050*	0.510^{ns}	0.749^{ns}	<0.001***	0.249^{ns}	0.389^{ns}	0.096^{ns}

^{abcd} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).
P values- ns: not significant, *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

5.3.1.3. Surface colour of pork sausages

The fat level did not influence the surface colour of SS sausages, however the surface redness (a*) was significantly affected by salt level (Table 5.4). Conversely, the L* and b* values of IW sausages were significantly influenced by the fat and salt levels, and a significant fat x salt interaction was observed. The a* values of IW sausages were also influenced by fat level. In comparison to the control, the addition of SS and IW seaweeds to sausage formulations reduced ($P<0.05$) the lightness (L*) and redness (a*) of fresh pork sausages (Table 5.4). However, no significant effect on surface yellowness (b*) was observed. Other than the fat and salt levels having effects on the colour changes of SS and IW sausages, colour changes are also presumably due to pigments such as fucoxanthin, β- carotene, chlorophyll a and phaephytin a present in SS and IW (Ferraces-Casais *et al*., 2012). Similarly, the incorporation of sea spaghetti and konjac into frankfurters and sea spaghetti in gel/emulsion systems decreased L* and a* values (Jiménez-Colmenero *et al*., 2010; Cofrades *et al*., 2008). The negative a* values of IW sausages indicated an associated greenness in pork sausage treatments. This may be due to the higher chlorophyll a, which is predominant in green plants, present in IW (172mg/100g) than in SS (150.14mg/100g) (Einarsdóttir *et al*., 2022; Ferraces-Casais *et al*., 2012). The b* values for treatments IW 20f/0.5s and 15f/0.5s treatments were similar ($P>0.05$) to the control, while other treatments had lower ($P<0.05$) b* values (Table 5.5). Kim *et al*. (2010) also reported a reduction in the lightness of sausages with increasing levels of sea tangle. These authors concluded that brown seaweed pigments such as

xanthophyll, carotenes and chlorophyll have adverse effects on the colour of fresh, uncooked sausages.

Table 5.4. Surface colour (CIE L*a* b*) of reduced fat and salt pork sausages containing brown seaweeds.

Treatment	Sea spaghetti (SS)			Irish wakame (IW)		
	L*	a*	b*	L*	a*	b*
C25f/2s	70.65±0.55^{a}	7.52±0.54^{a}	14.63±0.72^{a}	73.41±0.56^{a}	6.29±0.32^{a}	13.26±0.23^{a}
20f/1.5s	66.28±0.19^{b}	4.15±0.46^{b}	14.02±0.25^{a}	63.70±1.09^{bc}	-0.20±0.17^{bc}	11.25±0.40^{b}
20f/1.0s	64.61±2.17^{b}	3.81±0.60^{b}	13.61±0.70^{a}	61.77±0.63^{c}	-0.57±0.12^{c}	11.30±0.19^{b}
20f/0.5s	65.23±0.04^{b}	5.24±0.86^{b}	12.84±0.56^{a}	65.23±0.04^{b}	-0.44±0.27^{bc}	12.84±0.56^{ac}
15f/1.5s	64.79±0.46^{b}	4.53±0.88^{b}	13.36±0.62^{a}	61.41±0.71^{cd}	-0.18±0.12^{bc}	11.86±0.33^{bc}
15f/1.0s	65.44±0.43^{b}	4.42±0.56^{b}	13.70±0.44^{a}	61.24±0.42^{cd}	-0.13±0.18^{bc}	11.48±0.25^{b}
15f/0.5s	65.47±0.97^{b}	5.42±0.50^{b}	12.94±0.47^{a}	65.47±0.97^{b}	-0.30±0.29^{bc}	12.94±0.47^{a}
10f/1.5s	63.66±1.68^{b}	4.82±0.89^{b}	13.69±0.84^{a}	57.91±1.00^{e}	-0.08±0.12^{bc}	11.56±0.31^{b}
10f/1.0s	62.90±0.93^{b}	4.30±0.39^{b}	13.09±0.40^{a}	58.99±1.13^{de}	0.35±0.13^{b}	11.24±0.21^{b}
10f/0.5s	64.76±3.20^{b}	5.19±1.14^{b}	13.95±1.07^{a}	57.58±1.69^{e}	0.02±0.66^{bc}	11.35±0.43^{b}
Overall effects (P values) of variables (fat, salt and fat x salt interaction) on seaweed sausages' surface colour (CIE L*a* b*).						
Fat	0.065^{ns}	0.473^{ns}	0.712^{ns}	<0.001***	0.005**	0.003**
Salt	0.486^{ns}	0.015*	0.355^{ns}	<0.001***	0.633^{ns}	<0.001***
Fat x salt	0.544^{ns}	0.911^{ns}	0.136^{ns}	<0.001***	0.226^{ns}	0.004**

^{abcde} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

P values- ns: not significant, *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

5.3.1.4. Texture profile analysis (TPA) of cooked pork sausages

The overall cohesiveness of SS sausages was significantly influenced by the fat, salt levels and a fat x salt interaction was observed. The chewiness was influenced by the fat and salt levels. The springiness of both SS and IW sausages were affected by the salt level (Table 5.5). The hardness and springiness values of SS sausage treatments were not different ($P>0.05$) from the control. The SS20f/1.0s treatment had significantly lower cohesiveness, while SS10f/1.5s had significantly higher chewiness values than the control. The overall hardness and chewiness of IW sausages was significantly affected by the fat and salt levels, and a fat x salt interaction was observed. The hardness and chewiness of IW sausage treatments: 20f/1.5s, 20f/1.0s, 15f/1.5s and 10f/1.5s, were higher ($P<0.05$) than the control (Table 5.5). The springiness and cohesiveness of IW seaweed sausages were similar ($P>0.05$) to the control.

Cofrades *et al*. (2008) and Fernández-Martín *et al*. (2009) observed increasing hardness and chewiness and decreasing springiness by adding sea tangle in low-salt gel/emulsion meat systems and sea spaghetti in pork meat batter. However, some reports have observed softer patties generated by adding wakame seaweed (López-López *et al*., 2010). The variances in research findings are most likely attributed to seaweed type, seasonality of seaweed harvesting, and gross composition of seaweed utilised etc. Also, the reduction of fat in meat products such as meat batters and sausages can yield harder products (Tobin *et al*., 2013; Youssef and Barbut, 2011). In emulsified products, salt also acts as a solubilising agent, stabilising the fat within the protein matrix (protein–fat interaction) and contributing to juiciness and texture

(Keenan *et al*., 2014). According to Laranjo *et al*. (2016), reduced amounts of sodium can decrease the ability of protein to retain water, which leads to changes such as tougher and chewier products.

Table 5.5. Texture profile analysis (TPA) of reduced fat and salt pork sausages containing brown seaweeds.

Treatment	Sea spaghetti (SS)				Irish wakame (IW)			
	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N*mm)	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N*mm)
C25f/2s	43.07±4.30^{a}	0.881±0.02^{a}	0.707±0.01^{ab}	26.62±3.24^{a}	45.50±2.25^{a}	0.921±0.02^{a}	0.700±0.01^{ab}	29.34±1.29^{a}
20f/1.5s	47.65±4.26^{a}	0.893±0.04^{a}	0.685±0.02^{ab}	29.06±2.22^{ab}	59.73±2.94^{bc}	0.930±0.02^{a}	0.720±0.02^{b}	39.85±1.32^{c}
20f/1.0s	56.12±4.26^{a}	0.835±0.01^{a}	0.613±0.00^{c}	28.82±1.48^{ab}	61.24±2.89^{bc}	0.877±0.02^{a}	0.705±0.01^{ab}	37.81±1.90^{bc}
20f/0.5s	47.42±7.16^{a}	0.848±0.03^{a}	0.661±0.03^{bc}	26.34±2.09^{a}	45.51±2.95^{a}	0.874±0.05^{a}	0.668±0.01^{a}	26.64±2.34^{a}
15f/1.5s	55.12±6.33^{a}	0.898±0.01^{a}	0.692±0.00^{ab}	34.19±3.93^{ab}	59.35±5.12^{bc}	0.914±0.02^{a}	0.691±0.02^{ab}	37.39±2.16^{bc}
15f/1.0s	56.71±9.50^{a}	0.866±0.02^{a}	0.670±0.02^{ab}	32.86±5.62^{ab}	42.44±4.83^{a}	0.896±0.01^{a}	0.692±0.01^{ab}	26.13±2.06^{a}
15f/0.5s	49.94±8.37^{a}	0.872±0.02^{a}	0.674±0.03^{ab}	29.17±3.63^{ab}	43.92±5.41^{a}	0.884±0.02^{a}	0.694±0.01^{ab}	26.87±3.46^{a}
10f/1.5s	59.48±2.71^{a}	0.921±0.01^{a}	0.718±0.00^{b}	39.33±1.33^{b}	64.96±4.08^{c}	0.925±0.02^{a}	0.704±0.02^{ab}	42.30±2.29^{c}
10f/1.0s	52.21±2.75^{a}	0.855±0.05^{a}	0.684±0.03^{ab}	30.61±7.74^{ab}	51.05±2.95^{ab}	0.893±0.01^{a}	0.703±0.01^{ab}	32.00±1.55^{ab}
10f/0.5s	52.80±4.55^{a}	0.864±0.06^{a}	0.662±0.02^{bc}	30.44±5.13^{ab}	46.41±6.78^{a}	0.922±0.02^{a}	0.708±0.01^{b}	30.20±4.43^{a}
Overall effects (P values) of variables (fat, salt and fat x salt interaction) on seaweed sausages' TPA.								
Fat	0.414^{ns}	0.373^{ns}	0.004**	0.037*	0.009**	0.166^{ns}	0.157^{ns}	<0.001***
Salt	0.331^{ns}	0.013*	<0.001***	0.037*	<0.001***	0.009**	0.086^{ns}	<0.001***
Fat x salt	0.475^{ns}	0.918^{ns}	0.046*	0.451^{ns}	0.010**	0.274^{ns}	0.012*	0.006**

^{abc} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

P values- ns: not significant, *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

5.3.1.5. Sensory Analysis (Sensory acceptance testing (SAT)) on cooked pork sausages

The effects of fat, salt, fat x salt, treatment, batch, and panellist on SAT results is shown in Table 5.6. The overall SAT results of SS sausages were not influenced by the fat, salt levels and a fat x salt interaction was not observed. The treatment and batch did not have effects on the SS sausages' overall SAT results. However, the panellists had an influence on SS sausages' SAT descriptors, except aroma. Panellists did not perceive a difference between SS sausage formulations, and hedonic liking results were not significantly different from the control (Table 5.6). Therefore, all SS treatments were deemed acceptable by panellists. This result may be attributed to the concentration of sea spaghetti (2.5%) in the reformulated sausages masking the variation in fat and salt levels amongst treatments. Hence, the SS treatment with the lowest fat and salt levels (10f/0.5s) was selected for packaging and shelf-life analysis trials (lipid oxidation and microbiological analysis).

The overall SAT results, except aroma, of IW sausages were not influenced by the fat, salt levels and a fat x salt interaction was not observed. The aroma was influenced by the fat level. The treatment had an effect on the overall texture, flavour and overall acceptability of IW sausages. The random variable "batch" had no effect on SAT descriptors, while "panellist" influenced the SAT results (Table 5.6). The addition of IW to sausage formulations did not significantly affect the liking of appearance and aroma of reduced fat and salt sausages compared to the control. The texture and flavour sensory descriptors scores for 20f/0.5s, 15f/0.5s, 10f/1.0s and 10f/0.5s treatments were lower ($P<0.05$) than the control. Also, the texture descriptor score

for treatment 10f/1.5s was lower ($P<0.05$) than the control. Treatment 10f/1.0s had the lowest liking ($P<0.05$) for flavour and overall acceptability (Table 5.6). The IW sausage treatment 15f/1.0s contained the lowest fat and salt levels and was not significantly different from the control with respect to each sensory descriptor. Therefore, this IW treatment (15f/1.0s) was selected for the packaging and shelf-life study.

Generally, the sensory scores for IW sausages were lower than SS sausages. Vilar *et al*. (2021) reported a higher odour intensity for 1-Octen-3-ol in Irish wakame (3.3) compared to sea spaghetti (2) following gas chromatography olfactometry (GC-O) analysis. This compound has been shown to contribute to the formation of a seafood aroma (Van Durme *et al*., 2013), which may explain the reduced acceptability of IW containing pork sausages.

Table 5.6. Sensory acceptance testing (SAT) of reduced fat and salt pork sausages containing brown seaweeds.

Treatment	Sea spaghetti (SS)					Irish wakame (IW)				
	Appearance	Aroma	Texture	Flavour	Overall Acceptability	Appearance	Aroma	Texture	Flavour	Overall Acceptability
C25f/2s	6.91±2.02^{a}	7.25±1.68^{a}	7.24±1.92^{a}	7.57±1.54^{a}	7.18±1.76^{a}	6.58± 1.99^{a}	7.03±1.34^{a}	7.03±1.59^{a}	6.98±1.45^{a}	6.67±1.77^{a}
20f/1.5s	6.94±1.29^{a}	7.61±4.77^{a}	6.77±1.25^{a}	7.75±7.25^{a}	6.62±1.52^{a}	5.95±1.65^{a}	6.36±1.28^{a}	6.13±1.40^{ab}	6.26±1.30^{ab}	6.10±1.44^{ab}
20f/1.0s	6.95±1.48^{a}	8.46±8.02^{a}	6.40±1.92^{a}	6.97±1.58^{a}	6.78±1.66^{a}	5.92±1.59^{a}	6.48±1.26^{a}	5.57±1.50^{ab}	6.25±1.28^{ab}	5.73±1.31^{ab}
20f/0.5s	6.87±1.57^{a}	6.68±1.66^{a}	6.36±1.65^{a}	6.13±1.53^{a}	6.07±1.54^{a}	5.75±1.22^{a}	6.13±1.16^{a}	4.97±1.47^{b}	5.53±1.40^{b}	5.42±1.23^{ab}
15f/1.5s	6.89±1.36^{a}	8.39±7.21^{a}	6.49±1.68^{a}	6.80±1.32^{a}	6.73±1.33^{a}	6.09±1.46^{a}	6.11±1.56^{a}	5.73±1.46^{ab}	6.03±1.46^{ab}	5.80±1.44^{ab}
15f/1.0s*	6.82±1.65^{a}	6.66±1.97^{a}	6.15±2.07^{a}	6.73±1.76^{a}	6.49±1.68^{a}	**6.24±1.07^{a}**	**6.19±1.40^{a}**	**6.00±1.26^{ab}**	**5.89±0.99^{ab}**	**6.00±1.14^{ab}**
15f/0.5s	6.93±0.35^{a}	6.61±1.84^{a}	6.38±1.99^{a}	6.14±1.66^{a}	6.16±1.50^{a}	6.30±1.09^{a}	5.91±1.15^{a}	5.19±1.72^{b}	5.45±1.46^{b}	5.34±1.72^{ab}
10f/1.5s	6.96±1.31^{a}	6.78±1.74^{a}	5.85±1.84^{a}	6.39±1.58^{a}	6.35±1.74^{a}	5.90±1.39^{a}	5.88±1.27^{a}	5.38±1.68^{b}	5.85±1.22^{ab}	5.59±1.12^{ab}
10f/1.0s	6.97±1.58^{a}	6.76±1.83^{a}	5.99±1.77^{a}	6.59±1.78^{a}	6.46±1.75^{a}	6.17±0.94^{a}	6.13±1.45^{a}	4.89±1.58^{b}	5.45±1.27^{b}	5.21±1.23^{b}
10f/0.5s*	**7.09±1.38^{a}**	**6.76±1.40^{a}**	**5.97±1.78^{a}**	**6.10±1.84^{a}**	**5.94±1.69^{a}**	5.81±1.56^{a}	5.70±1.58^{a}	5.18±1.74^{b}	5.16±1.59^{b}	5.31±1.77^{ab}
Overall effects (P values) of fixed (fat, salt and fat x salt interaction and treatment) and random (batch and panellist) variables on seaweed sausages' sensory descriptors.										
Fat	0.639^{ns}	0.783^{ns}	0.198^{ns}	0.508^{ns}	0.459^{ns}	0.113^{ns}	0.034*	0.252^{ns}	0.773^{ns}	0.743^{ns}
Salt	0.990^{ns}	0.388^{ns}	0.923^{ns}	0.333^{ns}	0.356^{ns}	0.890^{ns}	0.650^{ns}	0.431^{ns}	0.325^{ns}	0.521^{ns}
Fat x salt	0.844^{ns}	0.572^{ns}	0.251^{ns}	0.435^{ns}	0.716^{ns}	0.752^{ns}	0.997^{ns}	0.120^{ns}	0.405^{ns}	0.216^{ns}
Treatment	0.921^{ns}	0.581^{ns}	0.053^{ns}	0.274^{ns}	0.051^{ns}	0.392^{ns}	0.062^{ns}	<0.001***	<0.001***	0.002**
Batch	0.557^{ns}	0.567^{ns}	0.926^{ns}	0.282^{ns}	0.638^{ns}	0.088^{ns}	0.971^{ns}	0.231^{ns}	0.612^{ns}	0.189^{ns}
Panellist	<0.001***	0.365^{ns}	<0.001***	0.024*	<0.001***	0.002**	0.011*	<0.001***	0.002**	<0.001***

^{ab} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

P values- ns: not significant, *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

*Seaweed treatments selected for shelf-life analysis.

5.3.2. Shelf-life stability of packaged selected pork sausage formulations

Factors such as a high fat content, comminuted structure and lack of thermal processing all render pork sausages susceptible to quality deterioration and spoilage by both lipid oxidation and microbial contamination. In the present chapter (and chapter 6), the sausage seasoning (see section 5.2.2) contains antioxidant (E301 – sodium ascorbate) and preservative (E223 – sodium metabisulphite) agents which serve to enhance sausage quality and shelf-life.

Shelf-life extension of processed meats such as pork sausages is complex due to the multi-functional properties of the ingredients used in product formulations. For example, salt is used positively as a flavour enhancer, binder and has preservative effects with respect to microbial growth. However, salt can also negatively impact lipid stability in processed meats by accelerating lipid oxidation, resulting in off-odours and flavours (Moroney *et al*., 2013). Similarly, dried ground seaweeds contain a range of functional ingredients and health-promoting bioactive compounds (López-López *et al*., 2009) which would enhance product quality from a technological perspective and increase consumer appeal. At the same time, seaweeds also contain minerals such as iron and copper (MacArtain *et al*., 2007) which are known to promote lipid oxidation in muscle foods.

In the current study, a number of packaging formats were examined in order to minimise the potential negative effects of ingredient reformulation on pork sausage quality and shelf-life. Based on the sensory analysis results, the control (C25f/2s) and two seaweed-containing sausage formulations (SS10f/0.5s and IW15f/1.0s) were stored in aerobic packaging (AP), 80% O_2: 20% CO_2 modified atmosphere packaging (MAP80/20), 70% N_2: 30% CO_2 (MAP70/30) and vacuum packaged (VP) conditions.

Shelf-life indicators examined included lipid oxidation and microbiological analysis of packaged sausage formulations.

5.3.2.1. Lipid oxidation

Lipid oxidation (TBARS) values of the control, SS10f/0.5s and IW15f/1.0s formulations stored under aerobic packaging (AP), modified atmosphere packaging (MAP) and vacuum packaged (VP) conditions over 27 days at 4 ^{o}C, are shown in Table 5.7). In the control treatment, on days 6, 13 and 20, AP stored samples had higher ($P<0.05$) levels of lipid oxidation than the other packaging systems used in this study. On day 27, MAP70/30 control samples had lower ($P<0.05$) TBARS values than AP stored and VP control sausage samples. In the SS10f/0.5s treatment group, no significant differences were observed between packaging conditions on days 1, 6, 13 and 20. In IW15f/1.0s sausage samples stored in MAP70/30, lower ($P<0.05$) TBARS values were observed than in AP stored and VP samples.

The lipid oxidation values of the control, SS10f/0.5s and IW15f/1.0s, ranged from 0.65 to 3.33 mg MDA/kg sausage, 0.70 to 1.36 mg MDA/kg sausage and 0.87 to 2.03 mg MDA/kg sausage respectively, stored under AP, MAP80/20, MAP70/30 and VP conditions. The minimum threshold required to detect negative changes in the organoleptic characteristics of meat products is approximately 2.0 mg MDA/kg of meat (Delgado-Pando *et al*., 2012). In the control (C25f/2.0s) group, AP, MAP80/20 and VP treatments exceeded this threshold on days 13, 27 and 20, respectively. MAP70/30 stored control samples were still below the threshold on day 27 of storage.

In SS10f/0.5s sausage samples, the threshold was not exceeded; however, IW15f/1.0s stored under AP condition exceeded this threshold on day 27. Storage of meat in high-oxygen atmospheres (up to 80% oxygen) is useful for maintaining colour but can degrade meat quality overtime (Clausen *et al*., 2009; Lund *et al*., 2007). A naturally occurring pigment known as myoglobin imparts a purplish colour in fresh meat. However, when myoglobin is exposed to air, it reacts with oxygen to generate bright red oxymyoglobin, which is the colour consumers associate with fresh meat. Further exposure to oxygen transforms oxymyoglobin into brownish metmyoglobin, which has a much lower consumer appeal despite not indicating spoilage. Nitrogen used in MAP70/30 reduces lipid oxidation and pack collapse (Narasimha Rao and Sachindra, 2002). Hence, MAP70/30-stored sausages had lower lipid oxidation values.

Table 5.7. Lipid oxidation (TBARS in mg MDA/kg sample) of control and selected seaweed sausage formulations during storage at 4 °C.

Treatment	Packaging	Storage time at 4 °C (days)				
		1	6	13	20	27
C25f/2.0s (Control)	AP	0.96 ± 0.01^{abcdx}	1.65 ± 0.07^{axy}	2.50 ± 0.79^{axy}	3.30 ± 0.71^{ay}	3.33 ± 0.95^{ay}
	MAP80/20	0.88 ± 0.03^{bcdex}	1.20 ± 0.15^{bcxy}	1.37 ± 0.10^{bcxy}	1.61 ± 0.35^{bcdxy}	2.07 ± 0.71^{aby}
	MAP70/30	0.67 ± 0.09^{ex}	0.65 ± 0.05^{fx}	0.72 ± 0.05^{cx}	0.71 ± 0.01^{dx}	0.76 ± 0.02^{bx}
	VP	0.89 ± 0.00^{bcdex}	1.12 ± 0.14^{bcdx}	1.46 ± 0.36^{bcx}	2.11 ± 0.81^{bxy}	3.02 ± 0.64^{ay}
SS10f/0.5s	AP	0.79 ± 0.06^{dex}	1.00 ± 0.02^{cdexy}	1.19 ± 0.19^{bcxy}	1.24 ± 0.15^{bcdxy}	1.36 ± 0.36^{by}
	MAP80/20	0.85 ± 0.12^{cdex}	0.94 ± 0.07^{cdefx}	1.06 ± 0.09^{bcx}	1.09 ± 0.28^{bcdx}	1.10 ± 0.28^{bx}
	MAP70/30	0.70 ± 0.04^{ex}	0.75 ± 0.05^{efxy}	0.88 ± 0.06^{bcz}	0.86 ± 0.06^{cdyz}	0.94 ± 0.01^{bz}
	VP	0.84 ± 0.08^{dex}	0.95 ± 0.04^{cdex}	1.01 ± 0.16^{bcx}	1.03 ± 0.14^{bcdx}	1.24 ± 0.49^{bx}
IW15f/1.0s	AP	1.15 ± 0.17^{ax}	1.69 ± 0.18^{ax}	1.71 ± 0.25^{abx}	1.93 ± 0.51^{bcx}	2.03 ± 0.50^{abx}
	MAP80/20	1.08 ± 0.07^{abcx}	1.14 ± 0.10^{bcdxy}	1.32 ± 0.06^{bcyz}	1.33 ± 0.10^{bcdyz}	1.49 ± 0.06^{bz}
	MAP70/30	0.87 ± 0.01^{cdex}	0.89 ± 0.03^{defx}	1.25 ± 0.14^{bcyz}	1.16 ± 0.02^{bcdy}	1.38 ± 0.05^{bz}
	VP	1.10 ± 0.07^{abx}	1.36 ± 0.12^{bxy}	1.69 ± 0.25^{abxy}	1.75 ± 0.23^{bcdxy}	1.91 ± 0.55^{aby}

^{abcdef} Effect of packaging: within each treatment, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

^{xyz} Effect of storage time (days): mean values (±standard deviation) in the same row bearing different superscripts are significantly different (P<0.05).

AP- Aerobic packaging, MAP80/20- 80% O_2 20% CO_2, MAP70/30- 70% N_2 30% CO_2, VP-vacuum packaging

5.3.2.2. Microbiological analysis

Salmonella and *E. coli* remained absent throughout the storage period in all sausage sample treatments (Table 5.8). No differences ($P>0.05$) in the TVC were observed between packaging for each treatment and storage day.

The Commission Regulation No. 2073/2005 set the acceptability limits applied to minced and mechanically separated meat examined at the end of the manufacturing process and are as follows: Total viable counts (TVC): < 5 x 10^6 CFU/ g ($\log_{10}$ 6) of the product; *E. coli:* < 500 CFU/ g ($\log_{10}$ 2) of product, and *Salmonella*: absent in 10 g of product throughout the shelf-life (FSAI, 2014). According to these limits, the control and seaweed sausages (SS10f/0.5s and IW15f/1.0s) stored under AP exceeded the limit of acceptability ($\log_{10}$ 6) on days 27 and 20, respectively (Table 5.8). These results suggest that salt reduction is a significant contributor to the microbial stability of seaweed containing sausages. The salt reduction was expected to increase spoilage without additional preservative factors applied i.e., packaging. Salt has a preservative effect on processed meat by lowering water activity, which helps control microbial growth. The reduction can have a negative impact on the shelf-life (Vilar *et al*., 2020). The factors involved in the preservative properties of NaCl include the direct toxicity of Cl−, removal of oxygen from the medium, sensitisation of the organisms to CO_2, and interference with the rapid action of proteolytic enzymes (Rockwell and Ebertz, 1924). On day 27 (end of storage), the TVC of each treatment stored under MAP80/20, MAP70/30, and VP did not exceed the acceptable limit. MAP and VP are known to greatly contribute to preserving and extending meat products' shelf-life.

The French guidelines states the acceptable limit for dried seaweed products as follows: TVC: $\leq 10^5$ CFU/ g ($\log_{10}$ 5) of the product, faecal coliforms: $\leq$ 10 and *Salmonella*: absent in 25 g of product throughout the shelf-life (CEVA, 2014). According to these guidelines for dried seaweed products, SS10f/0.5s stored under AP and MAP (MAP80/20 and MAP70/30) surpassed the recommended limits for TVC by day 13 and 27, respectively. While Irish wakame sausage (IW15f/1.0s) stored under AP, MAP80/20 and MAP70/30 reached this acceptable limit on days 6, 20 and 27. These results suggest that the high oxygen in the aerobic conditions accelerated bacteria growth.

Brown seaweeds, specifically SS, inhibits the proliferation of food spoilage and pathogenic bacteria (Cox *et al*., 2010). However, the brown seaweeds used in this study exhibited no antimicrobial properties in seaweed sausages. This suggests that in order to exploit the antimicrobial effect of brown seaweeds, seaweed extracts rather than the whole seaweed would need to be added.

Table 5.8. TVC (log_{10} CFU/g) of control and selected seaweed sausage formulations during storage at 4 °C.

Treatment	Packaging	Storage time at 4 °C (days)				
		1	6	13	20	27
C25f/2.0s (Control)	AP	4.09±0.03^{ax}	4.27±0.05^{axy}	5.01±0.09^{axy}	5.50±0.68^{ay}	6.88±0.81^{az}
	MAP80/20	4.06±0.26^{ax}	4.15±0.05^{axy}	4.18±0.68^{axy}	4.22±0.65^{axy}	5.44±0.56^{ay}
	MAP70/30	4.08±0.50^{ax}	4.20±0.87^{ax}	4.16±0.83^{ax}	4.35±0.73^{ax}	5.11±0.97^{ax}
	VP	4.18±0.04^{ax}	4.18±0.10^{ax}	4.09±0.08^{ax}	4.89±1.18^{ax}	5.16±0.26^{ax}
SS10f/0.5s	AP	4.10±0.21^{ax}	4.80±1.37^{ax}	5.30±1.29^{ax}	6.56±1.48^{ax}	6.23±0.10^{ax}
	MAP80/20	4.09±0.23^{ax}	4.01±0.34^{ax}	4.14±0.94^{ax}	4.59±1.62^{ax}	5.34±1.26^{ax}
	MAP70/30	4.10±0.78^{ax}	4.08±0.07^{ax}	4.12±0.30^{ax}	4.12±0.62^{ax}	5.29±1.14^{ax}
	VP	4.03±0.23^{ax}	4.05±0.23^{ax}	4.12±0.55^{ax}	4.35±0.18^{ax}	4.85±0.43^{ax}
IW15f/1.0s	AP	4.13±0.12^{ax}	5.00±1.06^{axy}	5.25±0.01^{axy}	6.54±0.15^{ay}	6.38±0.14^{ay}
	MAP80/20	4.06±0.11^{ax}	3.97±0.15^{ax}	4.54±1.62^{ax}	5.09±1.57^{ax}	5.08±1.71^{ax}
	MAP70/30	4.10±0.87^{ax}	3.97±0.16^{ax}	4.08±0.16^{ax}	4.54±0.55^{ax}	5.05±0.04^{ax}
	VP	4.07±0.12^{ax}	4.07±0.18^{ax}	4.05±0.28^{ax}	4.14±0.20^{ax}	4.60±0.51^{ax}

[a] Effect of packaging: within each treatment, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

[xyz] Effect of storage time (days): mean values (±standard deviation) in the same row bearing different superscripts are significantly different (P<0.05).

AP- Aerobic packaging, MAP80/20- 80% O_2 20% CO_2, MAP70/30- 70% N_2 30% CO_2, VP-vacuum packaging

5.4. Conclusions

The comprehensive analysis conducted in this study offers valuable insight into the incorporation of brown seaweeds, sea spaghetti (SS) and Irish wakame (IW), into reduced fat and salt pork sausages, shedding light on various physicochemical, sensory and shelf-life properties. The proximate composition results showed significant impacts of fat and salt levels, as well as seaweed inclusion, on the protein, fat, moisture and ash contents of the sausages. SS and IW inclusion did not influence the pH but affected the salt content and WHC. Texture profile analysis highlighted variations in hardness, chewiness, and springiness depending on the fat and salt levels, with brown seaweed treatments generally exhibiting higher hardness and chewiness. Sensory analysis provided valuable insights into consumer acceptance, with SS treatments generally well-accepted by panellists, while IW treatments exhibited lower sensory scores, possibly due to differences in odour intensity between seaweed types. Hence, the selected sausage formulations using SS and IW were 10% fat, 0.5% salt (SS10f/0.5s) and 15% fat, 1% salt (IW15f/1.0s), respectively. Shelf-life stability was evaluated through lipid oxidation and microbiological analysis under different packaging conditions. Lipid oxidation results indicated that the MAP70/30 packaging proved to be the most effective, while microbiological analysis indicated that seaweed treatments stored under vacuum packaging condition generally remained within acceptable limits throughout the storage period. Overall, results from this study highlights the suitability of SS and IW as an ingredient to enhance nutritional value by reducing fat and salt, introduce unique sensory attributes and create novel variations of pork sausages. Future research may explore the use of other seaweed types, i.e. red seaweeds, and additional packaging

techniques. The use of these brown seaweeds should also be explored in other meat products such as white and black puddings.

Chapter 6.

Physicochemical, sensorial and shelf-life properties of pork sausages containing red Irish seaweed species (dulse- *Palmaria palmata* and nori- *Porphyra umbilicalis*) as partial fat and salt replacers

Abstract

The objective of this study was to assess the effect of *Palmaria palmata* (dulse PP) and *Porphyra umbilicalis* (nori) at 1% and 2.5% respectively, on the physicochemical and sensory characteristics of reformulated reduced fat (20%, 15% and 10%) and salt (1.5%, 1% and 0.5%) pork sausages. Subsequently, shelf-life evaluation (lipid oxidation and microbiological analysis) was performed on selected formulations under aerobic (AP), MAP70/30 (70% N_2 :30% CO_2), MAP80/20 (80% O_2 :20% CO_2) and vacuum (VP) conditions. Compared to the control, 15% and 10% fat PP sausages and 10% fat nori sausages had significantly higher protein content. PP and nori sausages had significantly higher moisture and lower fat contents than the control. Seaweed sausages with 1.5% salt had similar ash content to the control. Relative to the control, the addition of red seaweeds had no effect ($P<0.05$) on the WHC and TPA of seaweed sausages. PP and nori sausage formulations were darker than the control. In general, the findings from the sensory study indicate that seaweed sausages were positively received by the panellists, with PP sausages exhibiting superior performance compared to nori sausages. The selected sausage formulations using PP and nori were 10% fat, 0.5% salt (PP10f/0.5s) and 10% fat, 1% salt (nori10f/1.0s), respectively. The packaging, MAP70/30 (70% N_2 :30% CO_2) was the most effective packaging method used to extend the shelf-life (TVC and lipid oxidation) of selected reduced fat and salt, seaweed-formulated sausages.

6.1. Introduction

The demand for healthy meat products, such as those with reduced amounts of fat, calories, and salt, as well as those that contain additional beneficial ingredients, has surged (Olmedilla-Alonso *et al*., 2013). Although meat products contain beneficial minerals and vitamins (Banjari and Hjartåker, 2018), it is advised that red and processed meats such as pork meat and its processed derivatives can be consumed in moderation (Lorenzo *et al*., 2018). Pork meat and its processed products such as ham, bacon, puddings and sausages are widely consumed in Ireland. Excessive consumption of processed meats has been linked to numerous health issues, such as obesity, type 2 diabetes, cardiovascular diseases, and several types of cancer mainly due to their high fat and salt contents (Bellou *et al*., 2018; Boada *et al*., 2016; Rouhani *et al*., 2014; Zhang *et al*., 2017).

Salt and fat have specific roles in processed meats and to some extent, these benefits cannot be compromised in order to maintain the rheological, structural and nutritional properties, quality and acceptability of meat products (sausages). In an effort to reduce the incidence of illnesses caused by high salt consumption, the World Health Organization (WHO) issued a recommendation to limit the intake of salt (sodium chloride) to less than 5 g/day (equivalent to 2 g/day of sodium) in adults.

Total fat consumption should not exceed 30% of total energy intake; saturated fat intake should be less than 10% of total calorie intake, and trans-fat intake should be less than 1% of total energy intake, with a shift in fat consumption away from saturated fats and trans fats towards unsaturated fats, particularly polyunsaturated fats (WHO, 2020). Hence, a reduction in salt and fat in pork sausages, and

replacement with functional ingredients such as seaweeds may enhance the healthiness, quality and shelf-life of pork sausages.

Polysaccharides, omega-3 fatty acids, protein, carotenoids, phenolic compounds, vitamins, and minerals are among the nutrients and biocompounds present in seaweeds (Agregán *et al*., 2018; Agregán *et al*., 2017a; Agregán *et al*., 2017b; Gullón *et al*., 2020; Lorenzo *et al*., 2017). Numerous health benefits are associated with these phytonutrients, including antioxidant, antihyperlipidemic, antihypertensive, and anticancer activity (Cofrades *et al*., 2017; Zhao *et al*., 2018). Potassium, sodium, magnesium and calcium minerals are usually present in high amounts (Biswas *et al*., 2019) and can replace salty perception in low salt meat products. Amino acids such as arginine, present in red seaweeds can function as flavour enhancers and can be used to intensify the perceived saltiness in reduced salt products (Inguglia *et al*., 2017, Chapter 2). Mabeau *et al*. (1992) also reported that the high levels of aspartic and glutamic acids in seaweeds are responsible for a distinct umami flavour. In addition, Carmen (2009) noted that salty and umami tastes are complementary and can be adjusted to compensate for deficiencies in one or the other.

Polysaccharides, such as agar and carrageenan, are found in red seaweeds and make up around 70% of the cell wall contents. Another polysaccharide is cellulose and ranges from 7% to 24% (Barsanti and Gualtieri, 2014). Carrageenan has been utilized to replace fat in low-fat milk products in order to enhance textural, physicochemical, and sensory quality (He and Sebranek, 1996). Polysaccharides are frequently employed in low-fat meat products such as pork patties because of their ability to retain water and improve textural quality (Garcia-Cruz and Sacamparini, 1992).

Red seaweeds, *Palmaria palmata* (dulse PP) and *Porphyra umbilicalis* (nori), have been incorporated into meat products such as cooked ham and frankfurters (Barbieri *et al*., 2016; Vilar *et al*., 2020). Other red seaweeds (*Kappaphycus alvarezii* and *Eucheuma denticulatum*) have also been explored in chicken sausages for added nutrients (Babji *et al*., 2017; Biswas *et al*., 2019, 2020; Pindi *et al*., 2017). However, from an extensive review of the scientific literature, the addition of red seaweeds, dulse (*Palmaria palmata*) and nori (*Porphyra umbilicalis*), in simultaneously reduced-fat and -salt pork sausages has not been explored and merits investigation.

Therefore, the objective of this study was to investigate the effect of Irish red seaweeds, dulse (*Palmaria palmata*) and nori (*Porohyra umbilicalis*), as partial fat (20%, 15% and 10%) and salt (1.5%, 1.0% and 0.5%) replacers in fresh pork sausage formulations at a previously determined maximum acceptable inclusion level (1% and 2.5%, respectively) (Chapter 4). The physicochemical, sensory and the shelf-life stability (lipid oxidation and microbiological analysis) of selected pork sausage formulations stored in various packaging formats was also examined.

6.2. Materials and methods

6.2.1. Chemicals and reagents

Kjeltabs, sodium chloride (NaCl), hydrogen peroxide (H_2O_2), boric acid (H_3BO_3), hydrochloric acid (HCl) and sulphuric acid (H_2SO_4), Trichloroacetic acid (TCA) and 2-thiobarbituric acid (TBA) were supplied by Sigma- Aldrich Ireland Ltd., Vale Road, Arklow, Wicklow, Ireland. Silver nitrate and Potassium chromate (K_2CrO_4) were purchased from Fisher Scientific, United Kingdom. Potassium dichromate ($K_2Cr_2O_7$)

was supplied by BDH Limited Supplies, Poole, England. All solvents were of analytical grade.

6.2.2. Raw materials

Red seaweed species *Palmaria palmata* (dulse PP) and *Porphyra umbilicalis* (nori) were purchased from Wild Irish Seaweed, Co. Clare, Ireland. Seaweeds were hand-harvested off the coast of Co. Clare, Ireland, and were 100% naturally grown, sustainably harvested and organically certified. According to the supplier‘s specifications, all seaweeds were air-dried, dehumidified, and milled prior to purchase. The average particle size of red seaweeds was approximately 2.5 mm (Tyuftin *et al*., 2021).

Pork oyster muscle and pork backfat were supplied by Ballyburden Meat Processors (Cork, Ireland). Food-grade salt was obtained from British salt Ltd. (Cheshire, UK). A custom formulated reduced salt sausage seasoning (0.39% salt) containing rusk [wheat flour (calcium carbonate, Iron, Niacin, Thiamin), salt], sugar, dextrose, stabiliser (E450, E452), flavour enhancer (E621), preservative (E223), antioxidant (E301), natural flavourings, anticaking agent (E551), rapeseed oil, hydrolysed vegetable protein (soya, maise)] was obtained from Redbrook Ingredient Services (Mulhuddart, Dublin 15, Ireland). Rusk [Wheat flour (wheat flour, calcium carbonate, Iron, Niacin, Thiamin), salt, raising agent E503] and collagen casing were purchased from Viscofan UK Ltd. (Sevenoaks, Kent, UK).

6.2.3. Sausage manufacture

Fresh pork sausages (4.3 kg batches) (Control (C)) were formulated containing 41.1% pork oyster muscle, 25% pork back fat (f), 18.5% water (50:50, water: ice), 11.4% rusk and 2.0% seasoning and 2% salt (s) (0.39% salt from the sausage seasoning, 0.14% from the rusk plus 1.5% salt added as an ingredient into the formulation) (Table 6.1). The control formulation, designated C25f/2s, was based on the average composition of pork sausages typically available on the Irish market. Additional batches of pork sausages (n = 9) were manufactured with a sequential reduction of fat (f) (20%, 15% and 10%) and salt (s) (1.5%, 1% and 0.5%) levels (at each fat level) resulting in 20f/1.5s, 20f/1.0s, 20f/0.5s, 15f/1.5s, 15f/1.0s, 15f/0.5s, 10f/1.5s, 10f/1.0s and 10f/0.5s treatments. Seaweed species (PP or nori) were added to the additional pork sausage batch formulations at a level of 1.0% and 2.5%, respectively; as previously identified as the maximum acceptable inclusion level in pork sausages (Chapter 4).

Prior to sausage manufacture, pork oyster and backfat were minced through a 4 mm perforated plate (Talsabell SA., Valencia, Spain). Pork oyster, half of the water, salt, and the seasoning was mixed for 60 seconds at high-speed using a bowl chopper (Seydleman Bowl chopper, Burgstallstraße, Germany). The backfat and various seaweeds were added and mixed at high speed for 30 seconds. The remaining water was added, and the batter was mixed for an additional 30 seconds at high speed. Finally, the rusk was added and mixed at low speed for 30 seconds. Sausage batter formulations were stuffed using a piston-type sausage filler (Mainca UK Ltd., Berkshire, UK) into 21mm diameter collagen casings and hand-linked into individual pork sausages (~ 10 cm in length).

Table 6.1. Sausage treatments and formulations of sausages (%).

Treatment	Pork oyster		Pork backfat	Water	Rusk	Seasoning	Salt	Seaweed	
	PP	Nori						PP	Nori
C25f/2s*	41.1	41.1	25.0	18.5	11.4	2.0	2.0	0	0
20f/1.5s	45.6	44.1	20.0	18.5	11.4	2.0	1.5	1.0	2.5
20f/1.0s	46.1	44.6	20.0	18.5	11.4	2.0	1.0	1.0	2.5
20f/0.5s	46.6	45.1	20.0	18.5	11.4	2.0	0.5	1.0	2.5
15f/1.5s	50.6	49.1	15.0	18.5	11.4	2.0	1.5	1.0	2.5
15f/1.0s	51.1	49.6	15.0	18.5	11.4	2.0	1.0	1.0	2.5
15f/0.5s	51.6	50.1	15.0	18.5	11.4	2.0	0.5	1.0	2.5
10f/1.5s	55.6	54.1	10,0	18.5	11.4	2.0	1.5	1.0	2.5
10f/1.0s	56.1	54.6	10.0	18.5	11.4	2.0	1.0	1.0	2.5
10f/0.5s	56.6	55.1	10.0	18.5	11.4	2.0	0.5	1.0	2.5

* Control

6.2.4. Physicochemical properties

6.2.4.1. Proximate composition

The protein (Kjeldahl) and ash (muffle furnace at 550 ^{o}C) contents of sausage samples were analysed following AOAC Methods 954.01 and 942.05, respectively (AOAC, 1999). The moisture and fat content of sausages were determined using a SMART Trac system (CEM GmbH, Kamp- Lintfort, Germany). Results were expressed as g/100 g of sample.

6.2.4.2. Salt analysis

The salt content of sausage samples was determined by titration using silver nitrate (Kirk and Sawyer, 1991). The silver nitrate (0.1 N $AgNO_3$) solution was standardised against 0.1% sodium chloride (NaCl). Sausage samples were ashed at 550 ^{o}C in a muffle furnace as described in section 6.2.4.1. Ash samples were subsequently washed into conical flasks with 20 ml distilled water. The indicator (potassium

chromate and potassium dichromate) (2 ml) was added and standardised silver nitrate was used to titrate the solution from a clear yellow colour to an opaque light orange. Blank titrations were performed using 20 ml distilled water. Salt content was calculated using the following equation:

$$\% \text{ salt} = \frac{\text{Titre for sample (ml)} - \text{Titre for blank (ml)}}{\text{Mass of sample (g)}} \times \text{Molarity of } AgNO_3 \times 5.844$$

6.2.4.3. pH determination

Fresh sausage samples (5 g) were homogenised for 1 min at 24,000 rpm in 45 ml distilled water using an Ultra Turax T25 homogeniser (Janke and Kunkel, IKA-Labortechnik, GmbH and Co., Staufen, Germany). The pH was measured at 20 ^{o}C using a pH meter (Seven Easy portable, Mettler-Toledo GmbH, Schweizenbach, Switzerland).

6.2.4.4. Instrumental colour analysis

The colour of fresh sausage samples was measured according to the CIE L*a*b* colour system. The samples were allowed to equilibrate at room temperature for 30 minutes before measuring. The surface colour was measured using a Minolta chromameter (CR400, Minolta Camera Co.Ltd, Osaka, Japan) with an 11mm-diameter aperture and D65 illuminant. The chromameter was calibrated using a white tile (L=97.79, a=-0.11, b= 2.69).

6.2.4.5. Texture profile analysis

The texture profile analysis (TPA) of sausage samples was carried out using a texture analyser TA-XF10S1.5i (Stable Micro System, Surrey, England) based on a method described by Bourne (1978). Cooked sausage samples were allowed to equilibrate at room temperature for 30 minutes before measuring. The samples were cut into cylindrical slices (10 mm thickness), and four pieces were measured per sausage sample. The samples were subjected to a two-cycle compression using a 30 kg load cell. All samples were compressed to 40% of their original height using a cylindrical probe (SMP/35 compression plate) at a cross-head speed of 1.5 mm/s. Determining factors include; hardness (N), the maximum force required for the first compression; springiness (mm), distance the sample recovers after initial compression; cohesiveness (dimensionless), the ratio of positive force area during the second compression and chewiness (N*mm), the product of gumminess and springiness.

6.2.4.6. Water holding capacity

The water holding capacity (WHC) was measured using the method described by Lianji and Chen (1989). Fresh sausage samples (10 g) were weighed into glass jars and heated in a water bath for 10 min at 90 ^{o}C. After heating, the samples were removed, wrapped in cheesecloth, and placed in 30 ml centrifuge tubes lined with cotton wool at the bottom of each tube. The samples were centrifuged (Avanti J-E centrifuge, Beckman Coulter, California, USA) at 13,300 g for 10 min at 4 ^{o}C. After centrifugation, the cheesecloth was removed, and samples were reweighed. WHC was calculated using the following calculation:

$$\% \text{ Water holding capacity} = 1 - \frac{(B-A)}{(B \times M)} \times 100$$

B is the sample's weight before heating, A is the weight of the sample after heating and centrifuging, and M is the % moisture of the sample.

6.2.4.7. Cook loss

Sausage weight was recorded before and after cooking. Sausages were cooked in a Zanussi oven at 180 ^{o}C for 22 min until an internal temperature of 72 ^{o}C was reached. Cook loss was calculated using the following equation:

$$\% \text{ Cook loss } = \frac{\text{Raw weight-cooked weight}}{\text{Raw weight}} \times 100$$

6.2.5. Sensory Analysis (Sensory acceptance testing)

Sensory acceptance testing (SAT), also known as affective testing or hedonic testing, is a sensory technique used to assess product acceptability and liking/preference or its unique sensory properties (Cardello and Jaeger, 2010).

Sausages were cooked as described in section 5.2.4.7, cooled and cut into cylindrical pieces (~2 cm). sausage samples were placed on plates and randomly assigned three digit codes. Sensory acceptance testing (hedonic (liking)) was carried out using the same set of untrained consumer panel (n=25) from chapter 5 section 5.2.5, (different set of panellists from chapter 4), who were familiar with the taste of pork sausages (Stone *et al*., 2012). Sensory analysis was conducted in sensory booths conforming to international standards (ISO, 2014) at room temperature. The assessors were presented 20 sausages samples (10 treatments x 2 replicates). This was carried out over two sessions, with the same set of panellists (n=25), to reduce panellist fatigue. Water for palate cleansing between samples was also provided. Assessors were asked to indicate their scores in relation to the following hedonic terms; appearance,

aroma, flavour, texture, and overall acceptability on a 10 cm hedonic scale (1= extremely dislike and 10=extremely like). All subjects gave informed consent for inclusion before participating in the sensory study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the University College Cork Social Research and Ethics Committee (Log 2021-188).

6.2.6. Packaging and shelf-life of pork sausages

Optimum packaging conditions for the control (C25f/2s) and two selected sausage formulations PP10f/0.5s and nori10f/1.0s, was investigated using four packaging treatments. Sausage samples were placed in low oxygen permeable (< 1cm^3/m^2/24 h at STP) polystyrene/ethyl vinyl alcohol/polyethylene trays and over-wrapped with oxygen-permeable film (cling film) for aerobic packaging (AP) conditions. Using modified atmosphere packaging (MAP) technology, trays containing the sausage treatments were flushed with 80% O_2: 20% CO_2 (MAP80/20) or 70% N_2: 30% CO_2 (MAP70/30) 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) (Salami *et al*., 2020).

Sausage samples were also placed in vacuum pouches (polyamide/ polyethylene 90, O_2: 50 cm^3 /m^2 /24 h STP, N_2: 10 cm^3 /m^2 /24 h STP, CO_2: M150 cm^3 /m^2 /24 h STP) and vacuum packaged (VP) using a vacuum machine (Henkelmann, Polar 80, Henkelmann, Titaniumlaan 10, 5221 CK's-Hertogenbosch, Netherlands). All packaging treatments (AP, MAP80/20, MAP70/30 and VP) were stored for up to 27 days at 4 ^oC. Shelf-life measurements included lipid oxidation and microbiological analysis.

6.2.6.1. Lipid oxidation

Lipid oxidation measurements were carried out following the method described by Siu and Draper (1978) on aerobically packaged (AP), MAP80/20, MAP70/30 and vacuum packaged (VP) sausages on days 1, 6, 13, 20 and 27 stored at 4 ^{o}C. Results were expressed as TBARS in mg malondialdehyde (MDA)/kg sausage.

6.2.6.2. Microbiological analysis

Microbiological analyses (Total viable counts (TVC), *Escherichia coli (E. coli)* and *Salmonella*) were determined as described by (O' Neill *et al*., 2018). Analyses were carried out on AP, MAP80/20, MAP70/30 and VP sausages on days 1, 6, 13, 20 and 27 stored at 4^{o}C. Except for *Salmonella*, the results were given as $\log_{10}$ colony-forming units per gram of sausage (CFU/g)- See section **5.2.6.2.**

6.2.7. Statistical analysis

Statistical analysis was carried out using the IBM SPSS 28.0 for Windows (SPSS, Chicago, IL, USA) software package. All analyses were performed in duplicate and three independent experimental trials were carried out. The physiochemical data of seaweed sausages was analysed using a two-way ANOVA to investigate the effects of salt level, fat level and fat x salt interactions. Tukey's post-hoc test was used to adjust for multiple comparisons between treatment means when significance was detected at $P<0.05$.

A mixed model ANOVA was used to analyse the sensory analysis data with treatment as a fixed effect and batch and panellist as random effects. Interactions between

fixed and random effects were measured and Tukey's post-hoc test was used to adjust for multiple comparisons between treatment means.

In the packaging study (lipid oxidation and microbiological analysis), a full repeated measures ANOVA was conducted to investigate the effect of packaged sausage treatments and storage time on lipid oxidation and microbial growth. Sausage treatment represented the 'between-subjects' factor and the effect of time was measured using the 'within-subjects' factor. Tukey's post-hoc test was used to adjust for multiple comparisons between treatment means when significance was detected at $P<0.05$.

6.3. Results and Discussion

6.3.1. Physicochemical properties of sausage formulations

6.3.1.1. Proximate composition

The proximate composition of sausages containing dulse (PP) and nori is shown in Table 6.2. The overall protein content of PP sausages was influenced by the salt level and a significant fat x salt interaction was observed, while nori sausages were influenced by the fat level only. The overall moisture content of PP sausages was influenced by fat, salt levels and a fat x salt interaction was observed, while nori sausages was influenced by the fat and salt levels. For a given fat level (20%, 15% and 10%), within each PP and nori treatment, there were no significant differences in the protein, moisture and fat content of pork sausages containing red seaweeds. In relation to the control, PP sausage treatments containing 15% and 10% fat and nori sausage treatments containing 10% fat had higher ($P<0.05$) protein contents. The increased pork oyster content in seaweed sausage formulations (Table 6.1), may

account for this increased protein content of seaweed sausages. PP and nori seaweeds contain high protein content (19- 32%) (Chapter 3, section 3.3.1), and this may also have attributed to the increased protein content in pork sausages. Red seaweed sausage treatments had significantly higher moisture contents than the control. This is also as a result of the higher level of pork oyster added to these treatments (Table 6.1).

The overall fat content of PP sausages was influenced by the fat, salt levels and a fat x salt interaction was observed, while nori sausages were influenced by the fat level and a fat x salt interaction was observed. Sausage treatments PP and nori also had significantly lower fat contents than the control, due to reduced fat formulations (Table 6.1). Results from chapter 3, section 3.3.1 reported that seaweeds used in this study have low fat contents (1.02-1.52%) and at an inclusion level of 1% and 2.5%, contributed minimal fat to the various sausage formulations. The overall ash content of PP sausages was influenced by the fat, salt levels and a fat x salt interaction was observed and nori sausages were significantly affected by the salt level. PP and nori sausage treatments containing 1.5% salt had similar ($P>0.05$) ash content to their controls, with other treatments having lower ($P<0.05$) ash values. This is as a result of minerals in these red seaweeds contributing to the total ash content of the sausages. Widati *et al*. (2021) also noted that the addition of red seaweed, *Eucheuma cottonii*, contributed positively to the ash content of cooked Indonesian-style beef meatballs. Within each salt level (1.5%, 1.0% and 0.5%), for each PP and nori treatment, there were no significant differences in the ash levels.

Table 6.2. Proximate composition of reduced fat and salt pork sausages containing red seaweeds.

Treatment	Dulse (PP)				Nori			
	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Protein (%)	Moisture (%)	Fat (%)	Ash (%)
C25f/2s	12.08±0.52^{a}	51.71±0.18^{a}	20.93±0.27^{a}	2.53±0.03^{a}	12.69±0.46^{a}	53.08±0.11^{a}	20.05±0.64^{a}	2.59±0.15^{a}
20f/1.5s	13.75±0.79^{abc}	54.99±0.34^{b}	16.20±0.84^{b}	2.52±0.05^{a}	13.86±0.47^{ab}	54.56±0.61^{b}	16.97±1.40^{b}	2.56±0.09^{a}
20f/1.0s	13.59±0.99^{abc}	54.83±0.30^{b}	16.75±0.04^{b}	2.01±0.11^{b}	13.06±0.51^{a}	54.50±0.10^{b}	16.91±0.39^{b}	2.01±0.12^{b}
20f/0.5s	13.52±0.23^{ab}	55.23±0.48^{b}	16.71±0.67^{b}	1.39±0.12^{c}	13.27±0.17^{a}	55.69±0.74^{bc}	16.42±0.55^{b}	1.67±0.02^{c}
15f/1.5s	14.04±0.18^{bc}	56.68±0.51^{c}	13.13±0.36^{c}	2.35±0.02^{a}	13.65±0.24^{ab}	56.83±0.53^{bc}	13.22±0.85^{c}	2.54±0.02^{a}
15f/1.0s	14.02±0.40^{bc}	57.23±0.43^{c}	13.46±0.61^{c}	2.00±0.02^{b}	13.59±0.21^{ab}	56.23±1.32^{bc}	13.25±1.57^{c}	2.08±0.06^{b}
15f/0.5s	14.14±0.75^{bc}	57.63±0.44^{c}	13.10±0.38^{c}	1.51±0.02^{c}	13.66±0.69^{ab}	57.73±1.31^{cde}	13.23±1.15^{c}	1.64±0.00^{c}
10f/1.5s	15.01±0.61^{bcd}	60.07±0.44^{d}	9.76±0.30^{d}	2.51±0.02^{a}	14.64±0.60^{b}	59.28±0.72^{ef}	9.53±0.71^{d}	2.61±0.03^{a}
10f/1.0s	15.35±0.25^{cd}	60.33±0.71^{de}	9.31±0.23^{d}	2.01±0.06^{b}	14.77±0.46^{b}	59.03±1.21^{def}	10.11±0.52^{d}	2.09±0.01^{b}
10f/0.5s	16.01±0.90^{d}	60.89±0.22^{e}	9.13±0.62^{d}	1.55±0.11^{c}	14.75±0.24^{b}	60.55±0.14^{f}	10.07±0.34^{d}	1.53±0.00^{c}
Overall effects (P values) of variables (fat, salt and fat x salt interaction) on seaweed sausages' proximate composition.								
Fat	0.474^{ns}	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.098^{ns}
Salt	<0.001***	<0.001***	<0.001***	<0.001***	0.514^{ns}	0.002**	0.060^{ns}	<0.001***
Fat x salt	<0.001***	<0.001***	<0.001***	<0.001***	0.414^{ns}	0.886^{ns}	<0.001***	0.091^{ns}

^{abcdef} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).
P values- ns: not significant, *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

6.3.1.2. pH, salt, water holding capacity and cook loss

The pH of PP and nori seaweeds were 6.71 and 6.12, respectively (Chapter 3, section 3.3.1). The pH values of PP and nori sausage treatments ranged from 6.02 to 6.22 and 5.96 to 6.16, respectively (Table 6.3). The overall pH of PP sausages was significantly influenced by the fat and salt levels, while the pH of nori sausages was influenced by the salt level and a fat x salt interaction was observed. PP sausage treatments containing 0.5% and 1% salt had increased ($P<0.05$) pH values, compared to the control, while all nori sausage treatments had higher ($P<0.05$) pH values than the control. The addition of *Eucheuma cottonii*, a red seaweed, also increased the pH values of meatballs (Widati *et al*., 2021). The pH increasing effect of seaweed powder previously reported to be due to the presence of alkaline minerals such as sodium, potassium, calcium and magnesium (Fitri, 2012). Although within each fat treatment, the pH value of PP and nori sausage treatments decreased with increasing salt level. Poulanne *et al*. (2001) also observed a reduction in pH values as the amount of salt applied increased in pork sausage batter. The effect of salt on the pH of meat is most likely caused by the fact that the Cl^- ion is more strongly bound to the proteins (such as myosin) that the Na^+ ion. The adsorption of Cl^- ions with positively charged groups of myosin results in a shift of the isoelectric point; hence, lowering pH (Desmond, 2006; Hamm, 1986). This explains the inverse relationship between the pH and salt content of seaweed sausages.

The overall salt content of PP sausage treatments was significantly influenced by the fat, salt levels and a fat x salt interaction was observed, while the salt level had a significant effect on the salt content of nori sausages only. Seaweed- containing sausage formulations had lower ($P<0.05$) salt levels (Table 6.1). The overall WHC of

PP sausages was influenced by the fat level. For nori sausages, the WHC was influenced by the fat and salt levels, and a fat x salt interaction was observed. Both PP and nori seaweed sausage treatments had similar ($P>0.05$) WHC values to the control (Table 6.3). According to Laranjo *et al*. (2016), reduced amounts of sodium decrease the protein's ability to retain water. PP and nori seaweeds used in this study contained 2080 mg/100g and 1710 mg/100g of sodium, respectively (Chapter 3, section 3.3.4). Therefore, the incorporation of red seaweeds into sausages compensated for the reduced salt levels in sausage formulations.

The overall cook loss of PP and nori sausages was significantly affected by the fat and salt levels, and a fat x salt interaction was observed. Sausage treatments containing PP 20f/0.5s, 15f/1.0s and 15f/0.5s, and nori sausage treatments 15f/0.5s and 10f/0.5s had an increased ($P<0.05$) cook loss compared to the controls. Ruusunen *et al*. (2003) noted that the reduction of salt in meat products increases cooking loss due to its water- and fat- binding properties.

Table 6.3. pH, salt, water holding capacity (WHC) and cook loss of reduced fat and salt pork sausages containing red seaweeds.

Treatment	Dulse (PP)				Nori			
	pH	Salt (%)	WHC (%)	Cook loss (%)	pH	Salt (%)	WHC (%)	Cook loss (%)
C25f/2s	5.97±0.06^{a}	1.84±0.03^{a}	61.77±3.73^{ab}	22.52±3.21^{a}	5.83±0.01^{a}	1.88±0.03^{a}	61.49±4.98^{a}	22.57±2.09^{ab}
20f/1.5s	6.02±0.02^{ab}	1.50±0.05^{b}	57.54±4.05^{a}	26.65±2.50^{abc}	6.00±0.03^{bc}	1.50±0.03^{b}	65.55±2.79^{a}	19.03±2.16^{a}
20f/1.0s	6.09±0.02^{bcd}	1.03±0.01^{c}	58.54±2.28^{ab}	27.68±3.10^{abc}	6.09±0.02^{de}	1.01±0.01^{c}	63.14±4.91^{a}	27.31±1.48^{bc}
20f/0.5s	6.22±0.01^{f}	0.57±0.01^{d}	61.76±3.05^{ab}	33.87±0.53^{d}	6.16±0.02^{ef}	0.57±0.03^{d}	64.07±4.99^{a}	27.96±2.32^{bcd}
15f/1.5s	6.03±0.02^{ab}	1.54±0.02^{b}	61.15±1.75^{ab}	24.57±0.98^{abc}	5.96±0.02^{b}	1.50±0.05^{b}	65.16±2.80^{a}	24.10±1.55^{abc}
15f/1.0s	6.10±0.03^{cd}	1.00±0.02^{c}	60.29±3.75^{ab}	28.72±0.65^{bcd}	6.10±0.03^{def}	1.03±0.01^{c}	65.81±3.08^{a}	26.92±1.20^{bc}
15f/0.5s	6.19±0.01^{ef}	0.56±0.02^{d}	58.63±1.28^{ab}	30.38±2.18^{cd}	6.16±0.05^{f}	0.51±0.02^{d}	66.56±1.48^{a}	33.34±0.71^{d}
10f/1.5s	6.03±0.02^{abc}	1.55±0.01^{b}	65.91±3.08^{b}	23.07±1.99^{ab}	6.02±0.01^{cd}	1.51±0.03^{b}	69.18±2.42^{a}	23.83±2.94^{abc}
10f/1.0s	6.07±0.02^{bc}	1.06±0.02^{c}	65.66±1.10^{ab}	26.57±1.42^{abc}	6.10±0.03^{ef}	1.01±0.02^{c}	68.53±3.00^{a}	27.72±2.98^{bcd}
10f/0.5s	6.15±0.01^{de}	0.59±0.01^{d}	63.11±2.96^{ab}	28.30±1.37^{abcd}	6.15±0.01^{ef}	0.50±0.01^{d}	63.06±2.40^{a}	28.66±1.16^{cd}
Overall effects (P values) of variables (fat, salt and fat x salt interaction) on seaweed sausages' pH, salt, WHC and cook loss.								
Fat	<0.001***	<0.001***	<0.001***	<0.001***	0.066^{ns}	0.148^{ns}	0.031*	0.008**
Salt	<0.001***	<0.001***	0.352^{ns}	0.002**	<0.001***	<0.001***	0.049*	<0.001***
Fat x salt	0.323^{ns}	<0.001***	0.060^{ns}	0.004**	<0.001***	0.071^{ns}	0.039*	0.039*

^{abcdef} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

P values- ns: not significant, *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

6.3.1.3. Surface colour of pork sausages

The overall lightness of PP and nori sausages was affected by the fat and salt levels, and a fat x salt interaction was observed. The lightness (L*) results (Table 6.4) showed that all PP and nori seaweed sausage treatments were darker ($P<0.05$) than the control, this was due to the addition of red seaweeds and the reduced fat content in sausage treatments. Hughes *et al*. (1998) also noted that fat reduction resulted in darker sausages.

The overall a* values of PP sausages was influenced by the fat level and nori sausages were influenced by the fat and salt levels, and a fat x salt interaction was observed. The b* values of PP sausages was affected the fat x salt interaction; while for nori sausages, the salt level affected the b* values and a fat x salt interaction was observed. PP sausage treatments 20f/1.0s, 20f/0.5s and 15f/1.5s had lower redness (a*) values than the control while treatments 20f/1.5s, 20f/1.0s, 15f/1.5s, 10f/1.0s and 10/f0.5s had lower ($P<0.05$) yellowness (b*) values than the control. Generally, PP sausage treatments had reduced a* and b* values when assessed against the control. In comparison to the control, nori sausage treatments had lower ($P<0.05$) a* and b* values.

Phycobilin (phycoerythrin and phycocyanin) is a pigment present in red seaweeds and can further be categorized as phycoerythrobilin (pink–red compound), phycocyanobilin (blue compound), phycourobilin (yellow compound), and phycoviolobilin (purple compound) (Kannaujiya *et al*., 2021). This pigment exerted various colour effects in seaweed sausages. In a recent study conducted by Munsu *et al*. (2021) using 2-6% of red seaweeds, the pigment, phycobilin, present in red seaweeds was also attributed to the colour changes in chicken sausages. Widati *et*

al. (2021) also noted a reduction in the L*a*b* values of meatballs with increasing addition of *Eucheuma cottonii* (2.5%, 5% and 7.5%). In reformulated products, changes in colour parameters have been linked to fat, water, and meat pigment contents (Pietrasik, 1999). In the current study, the water level was constant in all treatments. Hence, other than the addition of red seaweeds, the colour changes observed between seaweed sausages could be attributed to the variations in pork oyster and fat (Table 6.1).

Table 6.4. Surface colour (CIE L*a*b*) of reduced fat and salt pork sausages containing red seaweeds.

Treatment	Dulse (PP)			Nori		
	L*	a*	b*	L*	a*	b*
C25f/2s	73.16±0.36^{a}	6.72±0.07^{a}	13.49±0.16^{a}	71.34±0.17^{a}	7.86±0.08^{a}	14.75±0.43^{a}
20f/1.5s	67.42±0.17^{bc}	5.61±0.42^{ab}	11.96±0.26^{bc}	54.42±1.85^{c}	4.73±0.07^{bc}	8.79±0.65^{c}
20f/1.0s	68.61±0.48^{b}	5.26±0.36^{b}	11.88±1.15^{bc}	53.10±0.99^{cd}	4.31±0.40^{c}	8.60±0.82^{c}
20f/0.5s	65.23±0.04^{cde}	5.24±0.86^{b}	12.84±0.56^{ab}	65.23±0.04^{b}	5.24±0.86^{bc}	12.84±0.56^{b}
15f/1.5s	64.67±0.84^{de}	5.43±0.26^{b}	11.26±0.50^{c}	51.42±1.27^{de}	4.83±0.33^{bc}	8.17±0.13^{cd}
15f/1.0s	66.65±1.40^{bcd}	5.80±0.22^{ab}	12.31±0.20^{abc}	49.60±1.12^{ef}	4.54±0.10^{c}	7.52±0.81^{cd}
15f/0.5s	65.47±0.97^{cde}	5.72±0.48^{ab}	12.94±0.47^{ab}	65.47±0.97^{b}	5.72±0.48^{b}	12.94±0.47^{b}
10f/1.5s	63.90±1.08^{e}	6.38±0.53^{ab}	12.26±0.27^{abc}	49.19±0.45^{efg}	4.91±0.41^{bc}	7.72±0.38^{cd}
10f/1.0s	64.56±0.87^{de}	5.89±0.43^{ab}	11.63±0.34^{bc}	48.32±0.76^{fg}	5.05±0.06^{bc}	7.53±0.44^{cd}
10f/0.5s	64.42±0.33^{de}	6.34±0.09^{ab}	11.77±0.49^{bc}	46.41±0.84^{g}	4.44±0.45^{c}	6.58±0.51^{d}
Overall effects (P values) of variables (fat, salt and fat x salt interaction) on seaweed sausages' surface colour (CIE L*a*b*).						
Fat	<0.001***	<0.001***	0.464^{ns}	<0.001***	<0.001***	0.119^{ns}
Salt	<0.001***	0.383^{ns}	0.415^{ns}	<0.001***	<0.001***	<0.001***
Fat x salt	<0.001***	0.294^{ns}	<0.001***	<0.001***	<0.001***	<0.001***

^{abcdefg} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).
P values- ns: not significant, *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

6.3.1.4. Texture profile analysis (TPA) of cooked pork sausages

The overall hardness of nori sausages was significantly affected by fat and salt levels and the cohesiveness was affected by the fat, salt levels and a fat x salt interaction was observed. The overall chewiness of PP and nori sausages was affected by the fat and salt levels, respectively. There was no difference ($P>0.05$) in the hardness, springiness, cohesiveness and chewiness between red seaweed sausage treatments and the control (Table 6.5). However, within each PP and nori fat level, the hardness and chewiness decreased with reducing salt content. PP and nori sausage treatments 10f/1.5s had higher ($P<0.05$) hardness and chewiness than 10f/0.5s. As stated previously, reducing the amount of sodium (salt) in a product lowers the protein's capacity to hold water, which results in changes in both the product texture and yield (product remining after processing) (Laranjo *et al*., 2016).

Previous studies have shown that reduced fat frankfurters had a tougher texture than their higher fat counterparts (Barbut and Mittal, 1996; Bloukas *et al*., 1997). Carrageenan, a red seaweed polysaccharide, has been shown to increase the hardness of reduced fat pork patties, similar to that of high fat pork patties (Kumar and Sharma, 2004). Carrageenan has also been reported to improve cohesiveness of reduced- fat pork sausages because of its gelling and binding nature (Huffman *et al*., 1992). Giese (1992) also reported improved chewiness and springiness in the reduced fat meat products because of the gel-like nature of carrageenan. These may be the reasons red seaweed sausages TPA is comparable to that of the control.

Cellulose fibre, another red seaweed polysaccharide, has also been noted to improve texture without affecting organoleptic quality in low fat frankfurters (Barbut and

Mittal, 1996; Zhao *et al*., 2018). To conclude, the type of fibre and particle size of the added seaweed also affected the texture of the meat (Cofrades *et al*., 2008).

Table 6.5. Texture profile analysis (TPA) of reduced fat and salt pork sausages containing red seaweeds.

Treatment	Dulse (PP)				Nori			
	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N*mm)	Hardness (N)	Springiness (mm)	Cohesiveness	Chewiness (N*mm)
C25f/2s	46.57±5.53^{ab}	0.887±0.08^{a}	0.712±0.02^{a}	29.37±4.61^{ab}	41.24±7.61^{abc}	0.903±0.01^{ab}	0.718±0.02^{a}	26.53±3.95^{ab}
20f/1.5s	49.42±6.71^{ab}	0.903±0.01^{a}	0.670±0.00^{a}	29.57±2.52^{ab}	56.50±9.50^{c}	0.891±0.03^{ab}	0.720±0.01^{a}	34.59±1.48^{b}
20f/1.0s	49.04±3.84^{ab}	0.887±0.03^{a}	0.667±0.03^{a}	29.06±4.02^{ab}	53.99±3.60^{bc}	0.863±0.02^{ab}	0.691±0.01^{a}	33.65±6.80^{b}
20f/0.5s	43.77±5.14^{ab}	0.862±0.02^{a}	0.673±0.07^{a}	24.62±1.69^{ab}	42.47±5.77^{abc}	0.915±0.07^{b}	0.699±0.01^{a}	27.00±1.99^{ab}
15f/1.5s	51.06±4.96^{ab}	0.885±0.00^{a}	0.699±0.01^{a}	31.51±2.81^{ab}	52.97±2.16^{bc}	0.880±0.01^{ab}	0.726±0.01^{a}	33.89±0.77^{b}
15f/1.0s	44.78±4.80^{ab}	0.847±0.04^{a}	0.667±0.02^{a}	25.26±2.75^{ab}	50.49±5.34^{bc}	0.875±0.01^{ab}	0.692±0.02^{a}	30.31±3.27^{b}
15f/0.5s	41.18±3.86^{a}	0.853±0.04^{a}	0.676±0.06^{a}	24.17±6.12^{a}	47.87±3.38^{abc}	0.877±0.03^{ab}	0.666±0.03^{a}	27.80±0.25^{ab}
10f/1.5s	55.78±2.30^{b}	0.900±0.01^{a}	0.693±0.01^{a}	34.70±1.76^{b}	49.95±6.67^{bc}	0.924±0.01^{b}	0.724±0.02^{a}	33.38±5.44^{b}
10f/1.0s	49.22±4.32^{ab}	0.903±0.02^{a}	0.688±0.01^{a}	30.56±3.60^{ab}	39.26±3.93^{ab}	0.891±0.01^{ab}	0.717±0.00^{a}	25.03±2.44^{ab}
10f/0.5s	38.20±3.28^{a}	0.866±0.05^{a}	0.676±0.03^{a}	22.24±2.70^{a}	31.74±5.79^{a}	0.786±0.10^{a}	0.631±0.09^{a}	18.38±2.65^{a}
Overall effects (P values) of variables (fat, salt and fat x salt interaction) on seaweed sausages' TPA.								
Fat	0.118^{ns}	0.176^{ns}	0.262^{ns}	0.032*	0.022*	0.131^{ns}	0.013*	0.143^{ns}
Salt	0.071^{ns}	0.630^{ns}	0.529^{ns}	0.085^{ns}	0.011*	0.821^{ns}	0.013*	0.005**
Fat x salt	0.101^{ns}	0.403^{ns}	0.932^{ns}	0.111^{ns}	0.291^{ns}	0.242^{ns}	0.025*	0.154^{ns}

^{abc} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).
P values- ns: not significant, *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

6.3.1.5. Sensory Analysis (Sensory acceptance testing (SAT)) of cooked sausages

The effects of fat, salt, fat x salt, treatment, batch, and panellist on SAT results is shown in Table 6.6. The overall SAT results, except aroma of PP sausages was not influenced by the fat, salt levels and a fat x salt interaction was not observed; aroma was influenced by the fat level.

The treatment had an effect ($P<0.001$) on flavour of PP sausages. The texture of nori sausages was influenced by the fat level, and the overall acceptability was influenced by the fat, salt levels and treatment. In both PP and nori sausages, the batch did not influence the SAT results and the panellists affected ($P<0.001$) the SAT descriptors. PP sausages had similar ($P>0.05$) liking of appearance, aroma, texture, and overall acceptability to the control (high fat and salt sausages) (Table 6.6). Panellists rated liking of flavour of PP sausage formulations 15f/0.5s, 10f/1.5s and 10f/0.5s lower ($P<0.05$) than the control. Also, hedonic sensory parameters, appearance, aroma, texture, and flavour of nori sausages were not significantly different from the control. This may be attributed to the red seaweed flavours masking the reduction of fat and salt in seaweed sausages. Although, nori treatments 15f/0.5s and 10f/0.5s had lower ($P<0.05$) liking of overall acceptability relative to the control. A recent study reported a decrease in the sensory acceptance of seaweed- containing chicken sausages in terms of appearance, colour, aroma, taste, hardness, and overall acceptance (Munsu *et al*., 2021). Hence, seaweed treatments PP10f/0.5s and nori10f/1.0s were selected for further shelf-life analyses (lipid oxidation and microbiological analysis) as they had comparable SAT results with the control treatment.

Table 6.6. Sensory acceptance testing (SAT) of reduced fat and salt pork sausages containing red seaweeds.

Treatment	Dulse (PP)					Nori				
	Appearance	Aroma	Texture	Flavour	Overall Acceptability	Appearance	Aroma	Texture	Flavour	Overall Acceptability
C25f/2s	5.96±1.71^{a}	6.30±1.56^{a}	6.15±1.57^{a}	6.76±1.33^{a}	6.40±1.59^{a}	6.59±1.92^{a}	6.69±1.56^{a}	6.29±2.04^{a}	6.39±1.85^{a}	6.51±1.82^{a}
20f/1.5s	6.75±1.49^{a}	6.23±1.67^{a}	6.04±1.30^{a}	5.61±1.78^{ab}	6.14±1.29^{a}	5.55±1.68^{a}	6.43±1.17^{a}	6.25±1.30^{a}	5.86±1.26^{a}	6.10±1.21^{abc}
20f/1.0s	6.40±1.41^{a}	6.55±1.06^{a}	5.33±1.73^{a}	5.99±1.15^{ab}	5.89±1.37^{a}	5.56±1.70^{a}	6.42±1.22^{a}	5.92±1.66^{a}	5.81±1.35^{a}	5.85±1.28^{abc}
20f/0.5s	6.31±1.85^{a}	6.47±1.33^{a}	5.00±1.65^{a}	5.76±1.30^{ab}	5.84±1.13^{a}	5.82±1.61^{a}	6.18±1.40^{a}	5.71±1.39^{a}	5.89±1.31^{a}	5.62±1.12^{abc}
15f/1.5s	6.89±0.89^{a}	6.51±1.04^{a}	6.09±1.59^{a}	5.92±1.50^{ab}	6.22±1.12^{a}	5.56±1.63^{a}	6.21±1.11^{a}	6.10±1.58^{a}	5.79±1.56^{a}	6.01±1.28^{abc}
15f/1.0s	6.48±1.30^{a}	6.31±1.33^{a}	5.33±1.66^{a}	5.57±1.27^{ab}	5.45±1.30^{a}	6.14±1.43^{a}	6.35±1.18^{a}	5.90±1.48^{a}	6.24±1.27^{a}	6.22±1.28^{ab}
15f/0.5s	6.54±1.55^{a}	6.16±1.38^{a}	5.18±1.54^{a}	5.45±1.29^{b}	5.39±1.35^{a}	6.05±1.20^{a}	6.24±1.44^{a}	5.35±1.26^{a}	5.40±1.39^{a}	5.19±1.05^{bc}
10f/1.5s	5.99±1.45^{a}	5.77±1.43^{a}	5.00±1.57^{a}	5.34±1.42^{b}	5.46±1.16^{a}	5.59±1.73^{a}	6.23±1.39^{a}	5.13±1.53^{a}	5.71±1.44^{a}	5.68±1.35^{abc}
10f/1.0s*	6.54±1.31^{a}	6.11±1.28^{a}	5.60±1.49^{a}	5.83±1.29^{ab}	5.88±1.28^{a}	**5.90±1.17^{a}**	**6.18±1.34^{a}**	**5.34±1.29^{a}**	**5.81±1.26^{a}**	**5.70±1.06^{abc}**
10f/0.5s*	**6.60±1.37^{a}**	**6.38±1.22^{a}**	**5.25±1.47^{a}**	**5.24±1.47^{b}**	**5.40±1.23^{a}**	6.04±1.76^{a}	5.68±1.57^{a}	5.14±1.63^{a}	5.21±1.31^{a}	4.97±1.42^{c}
Overall effects (P values) of fixed (fat, salt, fat x salt interaction and treatment) and random (batch and panellist) variables on seaweed sausages' sensory descriptors.										
Fat	0.822^{ns}	0.040*	0.365^{ns}	0.652^{ns}	0.234^{ns}	0.177^{ns}	0.096^{ns}	0.003**	0.303^{ns}	0.030*
Salt	0.928^{ns}	0.956^{ns}	0.173^{ns}	0.786^{ns}	0.391^{ns}	0.637^{ns}	0.676^{ns}	0.511^{ns}	0.216^{ns}	0.023*
Fat x salt	0.273^{ns}	0.277^{ns}	0.071^{ns}	0.506^{ns}	0.156^{ns}	0.322^{ns}	0.717^{ns}	0.140^{ns}	0.266^{ns}	0.253^{ns}
Treatment	0.146^{ns}	0.464^{ns}	0.200^{ns}	<0.001***	0.380^{ns}	0.060^{ns}	0.366^{ns}	0.057^{ns}	0.083^{ns}	<0.001***
Batch	0.358^{ns}	0.820^{ns}	0.516^{ns}	0.576^{ns}	0.109^{ns}	0.227^{ns}	0.630^{ns}	0.119^{ns}	0.094^{ns}	0.067^{ns}
Panellist	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

^{abc} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05). Significance of regression coefficients; *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant). *Seaweed treatments selected for shelf-life analysis.

6.3.2 Shelf-life stability of packaged selected pork sausage formulations

Based on the sensory analysis results, the control (C25f/2s) and two seaweed-containing sausage formulations (PP 10f/0.5s and nori 10f/1.0s) were stored in aerobic packaging (AP), 80% O_2:20% CO_2 modified atmosphere packaging (MAP80/20), 70% N_2:30% CO_2 (MAP70/30) and vacuum packaged (VP) conditions. Shelf-life indicators examined included lipid oxidation and microbiological analysis of packaged sausage formulations.

6.3.2.1. Lipid oxidation

Lipid oxidation values (TBARS) of the control, PP10f/0.5s and nori10f/1.0s treatments stored under aerobic packaging (AP), modified atmosphere packaging (MAP) and vacuum packaged (VP) conditions over 27 days at 4 ^oC, are shown in Table 6.7. Kumar and Sharma (2004) reported TBARS values of reduced fat ground pork patties containing carrageenan stored under AP and VP to be lower than the high-fat control on day 0 of their study, which was due to the lower fat content which reduces the substrate for oxidation (Jo *et al*., 1999). However, on day 1 of storage in this study, reduced fat sausages containing red seaweeds, stored under AP and VP conditions had similar ($P>0.05$) TBARS value to the control stored under the same conditions. Red seaweeds in this study also contain minerals such as iron, sodium and copper (Chapter 3, section 3.3.4) that can increase the susceptibility of muscle foods to lipid oxidation (Moroney *et al*. 2013).

On day 1 of storage, MAP80/20 stored nori10f/1.0s sausage samples had higher ($P<0.05$) TBARS value than MAP80/20 control samples. On day 13, TBARS value of AP PP10f/0.5s was significantly lower than AP control samples. On days 20 and 27, AP

PP10f/0.5s and nori10f/1.0s had TBARS values lower (P<0.05) than AP control treatments. Also on day 27, vacuum packaged PP10f/0.5s and nori10f/1.0s sausages had lower (P<0.05) TBARS values than vacuum packed control samples. Within PP10f/0.5s treatment, there was no significant differences observed between packaging conditions on days 1, 13 and 20. While on day 6, MAP70/30 stored PP10f/0.5s sausage samples had lower (P>0.05) TBARS value than AP samples. On day 27, PP10f/0.5s sausage samples stored under MAP70/30 had TBARS value lower (P>0.05) than those stored under MAP80/20. Sausage treatment, nori10f/1.0s, stored in MAP70/30 had TBARS value lower (P<0.05) than that of MAP80/20 on day 1 and also had lower (P<0.05) TBARS value than AP, MAP80/20 and VP nori10f/1.0s sausages on day 6.

The minimum threshold required to detect negative changes in the organoleptic characteristics of meat products is 2.0 mg MDA per kg of meat (Delgado-Pando *et al*., 2012). Control samples stored under AP, in MAP80/20 and vacuum exceeded the minimum threshold (2.0 mg MDA/kg of meat) required to detect negative changes in the sausages on day 13, 27 and 20, respectively. MAP80/20 stored PP10f/0.5s sausage sample exceeded this limit on day 27. Similar to results in chapter 5, the high oxygen content in AP and MAP80/20 stored sausage samples elevated the TBARS values. Storage of meat in high-oxygen atmospheres, such as modified atmosphere containing up to 80% oxygen, is useful for colour protection but can increase susceptibility of meat to oxidation over time and hence, degrade meat quality (Clausen *et al*., 2009, Lund *et al*., 2007). Unlike nitrogen in MAP70/30, which decreases susceptibility of lipid oxidation and pack collapse (Narasimha Rao and Sachindra, 2002).

Table 6.7. Lipid oxidation (TBARS in mg MDA/kg sample) of control and selected seaweed sausage formulations during storage at 4 °C.

Treatment	Packaging	Storage time at 4 °C (days)				
		1	6	13	20	27
C25f/2.0s (Control)	AP	0.96±0.01^{abcx}	1.65±0.07^{axy}	2.50±0.79^{axy}	3.30±0.71^{ay}	3.33±0.95^{ay}
	MAP80/20	0.88±0.03^{bcdx}	1.20±0.15^{bcdexy}	1.37±0.10^{bxy}	1.61±0.35^{bcdxy}	2.07±0.71^{bcdy}
	MAP70/30	0.67±0.09^{dx}	0.65±0.05^{fx}	0.72±0.05^{bx}	0.71±0.01^{dx}	0.76±0.02^{ex}
	VP	0.89±0.00^{bcdx}	1.12±0.14^{cdex}	1.46±0.36^{bx}	2.11±0.81^{bxy}	3.02±0.64^{aby}
PP10f/0.5s	AP	1.01±0.09^{abcx}	1.31±0.27^{abcx}	1.40±0.41^{bx}	1.24±0.27^{bcdx}	1.11±0.25^{dex}
	MAP80/20	1.03±0.10^{abcx}	1.23±0.16^{bcdex}	1.11±0.03^{bx}	1.20±0.45^{bcdx}	2.34±0.21^{abcy}
	MAP70/30	0.78±0.02^{cdx}	0.90±0.03^{defy}	0.91±0.03^{byz}	0.97±0.03^{cdyz}	0.98±0.02^{efz}
	VP	1.03±0.11^{abcx}	1.22±0.22^{bcdex}	1.31±0.33^{bx}	1.21±0.14^{bcdx}	1.27±0.18^{cdex}
Nori10f/1.0s	AP	1.05±0.08^{abx}	1.41±0.16^{abcxy}	1.59±0.30^{abxy}	1.84±0.26^{bcyy}	1.88±0.16^{bcde}
	MAP80/20	1.15±0.19^{ax}	1.52±0.04^{aby}	1.43±0.11^{bxy}	1.57±0.05^{bcdy}	1.67±0.04^{cdey}
	MAP70/30	0.79±0.01^{bcdx}	0.87±0.02^{efx}	1.03±0.02^{by}	1.17±0.05^{bcdz}	1.21±0.07^{cdez}
	VP	1.03±0.10^{abcx}	1.29±0.09^{abcdxy}	1.53±0.18^{byz}	1.61±0.08^{bcdz}	1.75±0.12^{cdez}

^{abcdef} Effect of packaging: within each treatment, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).

^{xyz} Effect of storage time (days): mean values (±standard deviation) in the same row bearing different superscripts are significantly different (P<0.05).

AP- Aerobic packaging, MAP80/20- 80% O_2 20% CO_2, MAP70/30- 70% N_2 30% CO_2, VP-vacuum packaging.

6.3.2.2. Microbiological analysis

Salmonella and *E. coli* remained absent throughout the storage period in all samples. TVC results showed no differences (P>0.05) between packaging for each treatment and storage day.

The Commission Regulation No. 2073/2005 set the acceptability limits applied to minced and mechanically separated meat examined at the end of the manufacturing process and are as follows: Total viable counts < 5 x 10^6 CFU/ g ($\log_{10}$ 6) of product; *E. coli* < 500 CFU/ g ($\log_{10}$ 2) of product, and *Salmonella*: absent in 10 g of product throughout the shelf-life (FSAI, 2014). According to these regulations, the TVC of MAP80/20, MAP70/30 and VP control treatments did not exceed the limit of acceptability on day 27 (end of storage), while aerobic packaged (AP) control samples reached this limit by day 27. MAP and VP are known to greatly contribute to preserving and extending meat products' shelf life. AP PP10f/0.5s samples reached this limit on day 20, while MAP80/20 and VP samples were reached on day 27. Aerobic packaged, MAP80/20 and VP nori10f/1.0s samples reached the limit of acceptability on day 27. Although, MAP70/30 stored seaweed sausages were still below the limit of acceptability.

The French guidelines states the acceptable limit for dried seaweed products as follows: TVC: $\leq$ 10^5 CFU/ g ($\log_{10}$ 5) of the product, faecal coliforms: $\leq$ 10 and *Salmonella*: absent in 25 g of product throughout the shelf-life (CEVA, 2014). With regards to these guidelines for dried seaweed products, AP PP10f/0.5s and nori10f/1.0s exceeded this set limit on day 13, MAP80/20 and VP reached this limit on day 20, and MAP70/20 on day 27. These results suggest that the high oxygen in the aerobic conditions such as AP and MAP80/20 accelerated bacteria growth.

However, VP involves the absence of oxygen, VP seaweed sausages had high and comparable TVC results to MAP80/20 samples. Also, nitrogen gas, which was employed in MAP70/30, displaces oxygen, and influences the shelf-life of perishable goods such as fresh sausages indirectly by slowing the growth of aerobic spoilage microbes (Parry, 1993).

Table 6.8. TVC (log_{10} CFU/g) of control and selected seaweed sausage formulations during storage at 4 °C.

Treatment	Packaging	Storage time at 4 °C (days)				
		1	6	13	20	27
C25f/2.0s (Control)	AP	4.06 ±0.09^{ax}	4.24±0.06^{axy}	5.03±0.05^{axy}	5.48±0.69^{aby}	6.91±0.76^{az}
	MAP80/20	4.08±0.21^{ax}	4.14±0.04^{ax}	4.18±0.67^{ax}	4.15±0.52^{ax}	5.47±0.54^{ay}
	MAP70/30	4.08±0.50^{ax}	4.24±0.81^{ax}	4.16±0.83^{ax}	4.35±0.73^{abx}	5.11±0.97^{ax}
	VP	4.17±0.05^{ax}	4.18±0.10^{ax}	4.07±0.06^{ax}	4.85±1.10^{abx}	5.17±0.28^{ax}
PP10f/0.5s	AP	3.96±0.16^{ax}	3.88±0.18^{ax}	5.15±0.85^{axy}	6.89±0.61^{byz}	8.39±1.15^{az}
	MAP80/20	3.97±0.19^{ax}	3.86±0.23^{axx}	3.75±0.05^{a}	5.06±0.63^{aby}	6.14±0.22^{az}
	MAP70/30	3.94±0.82^{ax}	3.88±0.02^{ax}	3.83±0.09^{ax}	4.95±0.65^{abxy}	5.65±0.56^{ay}
	VP	3.89±0.03^{ax}	4.01±0.27^{ax}	4.39±0.66^{ax}	5.97±0.72^{aby}	6.11±0.13^{ay}
Nori10f/1.0s	AP	4.78±0.51^{ax}	4.40±0.74^{ax}	5.26±1.22^{axy}	5.87±0.66^{abxy}	7.95±1.55^{ay}
	MAP80/20	4.12±0.28^{ax}	4.16±0.40^{ax}	4.65±1.38^{ax}	5.31±1.84^{abx}	6.26±2.93^{ax}
	MAP70/30	4.03±0.81^{ax}	4.06±0.06^{ax}	4.11±0.02^{ax}	4.71±0.19^{abxy}	5.18±0.10^{ay}
	VP	4.18±0.16^{ax}	4.18±0.33^{ax}	4.02±0.32^{ax}	5.38±1.39^{abx}	6.21±1.31^{ax}

^{ab} Effect of packaging: within each treatment, mean values (±standard deviation) in the same column bearing different superscripts are significantly different (P<0.05).
^{xyz} Effect of storage time (days): mean values (±standard deviation) in the same row bearing different superscripts are significantly different (P<0.05).
AP- Aerobic packaging, MAP80/20- 80% O_2 20% CO_2, MAP70/30- 70% N_2 30% CO_2, VP-vacuum packaging

6.4. Conclusions

Fat and salt are known to influence overall quality and shelf-life of meat products. Excessive consumption of fat and salt has a negative impact on human health. The proximate composition revealed that the inclusion of red seaweeds dulse (PP) and nori resulted in higher protein and moisture contents, while fat and ash contents were generally lower compared to the control sausages. These changes can be attributed to the inherent composition of PP and nori, and the increased pork oyster content in the formulations. Seaweed treatments generally exhibited higher pH values and lower salt content, while WHC remained similar to the control. Surface colour analysis revealed that seaweed sausages were generally darker due to the addition of PP and nori, and reduced fat content. Texture profile analysis indicated that seaweed sausages had comparable texture properties to the control. This can be attributed to the gel-like nature of red seaweed polysaccharides such as carrageenan and cellulose, which improve texture without compromising organoleptic quality. Generally, sensory analysis demonstrated that seaweed sausages were well-accepted by panellists with PP sausages having better results than nori sausages. The selected sausage formulations utilising PP and nori were 10% fat, 0.5% salt PP10f/0.5s and 10% fat, 1.0% salt nori10f/1.0s, respectively. Shelf-life stability analysis revealed that the packaging condition MAP70/30 (70% N_2: 30% CO_2) was the most effective for lipid oxidation and TVC (total viable counts). Results from this study highlights the suitability of PP and nori as ingredients in reducing fat and salt in pork sausages, thus developing a healthier variation of pork sausages. Future research may focus on exploring additional packaging techniques, and the use of these seaweeds in other meat products such as chicken and turkey sausages

Chapter 7

Irish consumers' attitudes towards seaweed-containing sausages: using selected seaweeds as potential ingredients in salt and fat reduction strategies

Abstract

Consumer (n=100) acceptability study of reduced- fat and -salt levels in seaweed-containing (dulse, nori, sea spaghetti and Irish wakame) sausages (PP10f/0.5s, Nori10f/1s, SS10f/0.5s and IW15f/1s), in comparison to experimental (EC) and commercial (CC) controls (EC- 25% fat, 2% salt and CC-23% fat, 2.1% salt, respectively) was conducted. Panellists were asked to indicate their hedonic responses on a 10 cm continuous line-scale. Consumers were asked to rate the intensity of 9 basic descriptors (colour, hardness, juiciness, grainy, saltiness, seafood, herbal, off-flavours and aftertaste). Hedonic descriptors were positively correlated to EC and CC samples. They were also negatively correlated to seaweed samples. Sensory intensity results indicated that both commercial and experimental sausage samples were positively associated ($P<0.001$) with juiciness and saltiness. Reduced fat and salt sausage containing PP, nori and SS were positively associated with the intensity descriptors; colour, seafood flavour ($P<0.001$) and off-flavour ($P<0.01$) and negatively associated ($P<0.001$) with juiciness and saltiness. Reduced fat and salt IW sausages were positively correlated with colour, texture ($P<0.001$) and herbal flavour ($P<0.01$) and negatively associated ($P<0.05$) with juiciness. Hedonic results indicated that consumers disliked the reduced fat and salt seaweed sausages and Nori10f/1s was the least liked and acceptable formulation. Further experiments to improve the sensory acceptability of reduced- fat and -salt seaweed sausages are necessary.

7.1. Introduction

Fat and salt play various roles in sausages, contributing to the product's overall quality and functionality. However, consumption in high quantities of either ingredient can negatively impact human health, hence the need for reduced-fat and -salt meat products. Reduced-fat meat products are perceived to be healthier by consumers and hence, markets exist for low-fat meat products. However, such meat products may suffer in terms of key and traditional product quality attributes, especially in relation to juiciness and flavour owing to the lower intramuscular fat content (Chizzolini *et al*., 1999). Ingredients such as vegetable oils (Jiménez-Colmenero, 2007), whey protein concentrate (WPC) (Naga Mallika *et al*., 2009), long-chain oligosaccharide (inulin) products (Crittenden and Playne, 1996) and some chemically modified starches (Tharanathan, 2005) have been used as modifying fat replacement in an attempt to circumvent any detrimental changes in sensory characteristics. Reduced-salt products are also marketed as healthier. Potassium chloride (KCl) is probably the most common salt substitute in low- or reduced-salt/sodium foods (Hur *et al*., 2004; Ruusunen and Puolanne, 2005). However, this imparts bitterness and loss of saltiness at levels used in excess of 0.6% in recipes (Hathwar *et al*., 2012). Hence, the quest continues to identify a true salt-replacing ingredient.

Brown (sea spaghetti- *Himanthalia elongata* and Wakame- *Undaria pinnatifida*) and red (*Palmaria palmata*- dulse and *Porphyra umbilicalis*- nori) seaweeds have been incorporated into reduced-fat and or -salt meat products such as beef patties and frankfurters (Jiménez-Colmenero *et al*., 2010; López-López *et al*., 2011; Vilar *et al*., 2020). Seaweeds with bioactive compounds have been shown to offer health

benefits and can be used to reformulate meat products deemed "bad" by some consumers in an attempt to improve nutritional content (Gullón *et al*., 2020).

Previous studies which we have conducted in this research area have shown that it is possible to replace salt and fat in sausages without impacting negatively on the sensory characteristics of these products or impacting negatively on product shelf-life. What remains to complete this product development story is to ascertain the views of consumers as to the acceptability of these products from a marketing perspective. Hence, the objective of this study was to conduct an independent consumer acceptability study to ascertain if previously formulated (chapters 5 and 6) reduced-fat and salt- seaweed sausages which has found favour in experimental sensory trials would be accepted by a much larger consumer grouping.

7.2. Materials and methods

7.2.2. Raw materials

For consumer assessment of fat- and salt-reduced breakfast sausages employing seaweed replacement, all seaweeds utilised were sourced from Wild Irish Seaweed, Co. Clare, Ireland. According to supplier specifications, all seaweeds were air-dried, subsequently dehumidified, and milled to 1mm in size. Seaweeds were hand-harvested off the coast of Co. Clare, Ireland, and were 100% naturally grown, sustainably harvested and organically certified. Brown (*Himanthalia elongata* (Sea spaghetti SS), *Alaria esculenta* (Irish wakame IW)) and red ((*Palmaria palmata* (Dulse PP), *Porphyra umbilicalis* (Nori)) were used in this study.

Pork oyster muscle and pork backfat were supplied by Ballyburden Meat Processors (Cork, Ireland). Food-grade salt was obtained from British salt Ltd. (Cheshire, UK).

Reduced-salt sausage seasoning (Rusk [Wheat flour (calcium carbonate, Iron, Niacin, Thiamin), salt], Sugar, Dextrose, stabiliser (E450, E452), flavour enhancer (E621), preservative (E223, sodium metabisulphite), antioxidant (E301), Natural flavourings, anticaking agent (E551), rapeseed oil, hydrolysed vegetable protein (soya, maise)) with a total of 15.6% salt was obtained from Redbrook Ingredient Services (Mulhuddart, Dublin 15, Ireland). Rusk [Wheat flour (wheat flour, calcium carbonate, Iron, Niacin, Thiamin), salt (1.2%), raising agent E503] and collagen casing were purchased from Kiernan's Food Ingredients Ltd. (Dundalk Road, Carrickmacross, Co. Monaghan).

7.2.3. Sausage manufacture

Fresh pork sausages (4.3 kg batches) (Control (C)) were formulated containing 41.1% pork oyster muscle, 25% pork back fat (f), 18.5% water (50:50, water: ice), 11.4% rusk and 2.0% seasoning and 2% salt (s) (0.39% salt from the sausage seasoning, 0.14% from the rusk plus 1.5% salt added as an ingredient into the formulation) (Table 7.1). The experimental control formulation, EC25f/2s, was based on the average composition of pork sausages typically available on the Irish market. Additional batches of pork sausages (n = 4) were manufactured with reduced-fat and -salt, and various seaweed species (SS, IW and nori) were added at 2.5% and PP at 1.0% (Table 7.1). These inclusion levels were identified as the maximum acceptable inclusion level in pork sausages in thesis chapter 4.

Sausages were manufactured by mincing pork meat and backfat through a 4mm perforated plate (Talsabell SA., Valencia, Spain). Pork oyster, half of the water, salt, and the seasoning were mixed for 60 sec at high-speed using a bowl chopper

(Seydleman Bowl chopper, Burgstallstraße, Germany). The backfat and various seaweeds were added and mixed at high speed for 30 sec. The other half of the water was added, and the batter was mixed for 30 sec at high speed. Rusk was added and mixed at low speed for 30 sec. Sausage batter was stuffed using a piston-type sausage filler (Mainca UK Ltd., Berkshire, UK) into 21mm diameter collagen casings and hand-linked (~ 10 cm in length).

The optimum parameters for the formulation of sausage samples were determined by sensory and physicochemical analysis, as described in thesis chapters 3 and 4 and are shown in Table 7.1. An experimental control (EC) and commercial control (CC) were also employed for the analysis. The commercial sausage sample was purchased from Tesco, Ireland, which contained 60% pork oyster, 23% fat and 2.1% salt.

Table 7.1. Experimental control and reduced fat and salt seaweed sausages formulations (%).

Treatment	Pork oyster	Pork back fat	water	Rusk	Seasoning	Salt	Seaweed
EC25f/2s	41.1	25.0	18.5	11.4	2.0	2.0	0.0
PP10f/0.5s	56.6	10.0	18.5	11.4	2.0	0.5	1.0
Nori10f/1s	54.6	10.0	18.5	11.4	2.0	1.0	2.5
SS10f/0.5s	55.1	10.0	18.5	11.4	2.0	0.5	2.5
IW15f/1s	49.6	15.0	18.5	11.4	2.0	1.0	2.5

EC25f/2s- Experimental control 25% fat 2% salt, PP10f/0.5s- dulse 10% fat 0.5% salt, Nori10f/1s- nori 10% fat 1% salt, SS10f/0.5s- sea spaghetti 10% fat 0.5% salt, IW15f/1s- Irish wakame 15% fat 1% salt.

7.2.4. Sensory analysis (Consumer acceptability study)

The consumer study was carried out in the sensory science laboratory panel booths at University College Cork according to international standards (ISO 11136:2014). Consumers were recruited based on their availability and preference for meat products (sausages). The study took place over a couple of days, and allocated time slots were given to accommodate all required panellists (n=100). The following consumer hedonic descriptors were used. Participants were asked to indicate their degree of liking on a 10 cm continuous line-scale: liking appearance, flavour, texture, aroma and overall acceptability (OA). The untrained consumers were also asked to rate the intensity of important attributes, as described by Rolon *et al*. (2017). Although it is not traditional to collect intensity data from untrained panellists (Lawless and Heymann, 2010), gathering this information was desirable because it could help identify the consumer drivers of changes in liking, and the descriptors used were basic and easily understood. Recent work suggests that untrained consumers can provide meaningful intensity ratings for complex products (Li *et al*., 2014). Each participant answered the hedonic section first to avoid biasing the hedonic rating (Rolon *et al*., 2017).

Table 7.3 shows a list of intensity descriptors used, definitions and intensity scales. Intensity descriptors were also measured on a 10 cm continuous line-scale. The samples were served, coded in randomised order and presented simultaneously to consumers, who were instructed to use the water provided to cleanse their palates between tastings (Stone *et al*., 2020). All subjects gave informed consent for inclusion before participating in the consumer study. The study was conducted following the

Declaration of Helsinki, and the protocol was approved by the University College Cork Social Research and Ethics Committee (Log 2021-188).

7.2.5. Statistical analysis

Raw data obtained from consumer analysis was coded into Microsoft excel. ANOVA determined the relationship between the set of samples and consumer variables-partial least squares (APLS) regression using Unscrambler software (Unscrambler 10.3 CAMO Software ASA, Trondheim, Norway). The X-matrix was designed as 0/1 variables for sample treatments, and the Y-matrix contained consumer variables. Data were normalised during pre-processing by taking the logarithm to achieve uniform precision over the whole range of variation. Regression coefficients were analysed by Jack-knifing, which is based on cross-validation and stability plots (Martens and Martens, 2000). Principal component (PC) 1 versus PC 2 are presented; other PCs did not yield additional information. Statistical significance for the relationships analysed was defined as $P<0.05$-0.01 (significant), $P<0.01$-0.001 (highly significant) and $P<0.001$ (extremely significant).

Table 7.2. List of sensory (hedonic and intensity) descriptors and definitions used in consumer acceptability study of sausage samples.

Hedonic	Anchor points on scale	Definition
Appearance	Extremely dislike - extremely like	The liking of appearance
Flavour	Extremely dislike - extremely like	The liking of flavour
Aroma	Extremely dislike - extremely like	The liking of aroma
Texture	Extremely dislike - extremely like	The liking of texture
Overall acceptability	Extremely dislike - extremely like	The overall acceptability of the product
Intensity		
Colour	Light - dark	Assessment of internal colour. Degree of darkness.
Texture		
Hardness	Soft – firm	The resistance required to bite through the sausage using the front teeth
Juiciness	Dry - moist	Wet texture in the mouth
Grainy	None - extreme	Presence of particles
Flavour		
Saltiness	None – extreme	Taste sensation associated with salt
Seafood flavour	None – extreme	The flavour associated with seafood
Herbal flavour	None – extreme	The flavour associated with herbs
Off flavour	None – extreme	The distinct negative flavour associated with the product; is not seaweed or seafood flavour.
Aftertaste	None – extreme	Astringent or bitter

7.3. Results and Discussion

Consumer evaluators in this study consisted of 66% female and 44% male. The ages of assessors ranged from 19-58. The significance of estimated regression coefficients for the relationship between sensory terms and sausage samples are presented in Table 6.

For hedonic results, commercial sausage sample was positively correlated ($P<0.001$) to the liking of appearance, flavour, texture, aroma and overall acceptability. The experimental control was also positively associated with liking of appearance, flavour, texture, overall acceptability ($P<0.001$) and aroma ($P<0.01$). Hence, the liking of the experimental control is comparable to that of the commercial sample. Reduced-fat and -salt PP incorporated sausages were negatively associated with the liking of flavour, texture, aroma and overall acceptability ($P<0.001$). Nori sausages were negatively associated ($P<0.001$) with liking of appearance, flavour, texture, aroma and overall acceptability. Reduced-fat and -salt SS sausages samples were negatively correlated ($P<0.001$) to the liking of flavour, texture, aroma and overall acceptability. In contrast, reduced-fat and -salt IW sausages were negatively associated with the liking of appearance ($P<0.001$) and aroma ($P<0.05$). Vilar *et al*. (2020) also noted the negative impact of seaweed addition on the liking of appearance and aroma of frankfurters.

Sensory intensity results indicated that the commercial and experimental sausage samples were positively associated ($P<0.001$) with juiciness and saltiness. Hence, these samples were perceived to be moist and salty, which consumers generally prefer. Commercial samples were negatively associated ($P<0.001$) with texture,

grainy, seafood flavour, herbal flavour and off-flavour. These results imply that this sample was perceived as soft, no grain present and no seafood, herbal and off-flavours. Experimental controls were also negatively associated with colour, grainy, seafood, herbal ($P<0.001$), off-flavours, and after taste ($P<0.01$). Reduced-fat and -salt sausages containing PP were positively associated with intensity descriptors, colour, grainy, seafood flavour ($P<0.001$) and off-flavour ($P<0.01$). Nori (reduced-fat and -salt) sausages were positively correlated to colour, texture, grainy, seafood flavour, herbal flavour ($P<0.001$) and off-flavour ($P<0.01$). SS-reduced fat, and salt sausage samples were positively associated with colour, seafood flavour ($P<0.001$), grainy, off-flavour ($P<0.01$) and aftertaste ($P<0.05$). IW-reduced-fat and -salt sausage samples were positively associated with colour, texture ($P<0.001$) and herbal flavour ($P<0.01$). Reduced fat and salt seaweed sausages incorporated with PP, nori and SS were negatively associated ($P<0.001$) with juiciness and saltiness, while IW sausages were negatively associated ($P<0.05$) with juiciness only. Previous studies by Fellendorf *et al*. (2016a) and Vilar *et al*. (2020) also observed the effect of seaweed-based ingredients being perceived negatively on white pudding flavour and frankfurters, respectively.

From Figure 7.1, reduced fat and salt PP and SS sausage samples are situated in close proximity. This may be due to their fat and salt contents (10% fat and 0.5% salt) being similar, and thus, consumers (n=100) did not perceive much difference between these two samples, regardless of the seaweed-type. Overall, nori10f/1s was the least liked and acceptable seaweed sausage, which may be due to the low-salt content (5.35%) of nori seaweed quantified in thesis chapter 3, section 3.3.1. Mabeau *et al*. (1992) reported that the high levels of aspartic and glutamic acids in seaweeds are

responsible for their distinct flavour (umami flavour) and taste. Mišurcová *et al*. (2014) also indicated that alanine, glycine and glutamic acid were considered the main components of seaweed flavour. Of all four seaweeds used in this study, nori seaweed had the highest levels of aspartic, glutamic, alanine and glycine amino acids (Chapter 3, section 3.3.3). These high amino acids levels could also be a significant contributing factor to the low liking and acceptability of reduced-fat and -salt nori sausages (nori10f/1s).

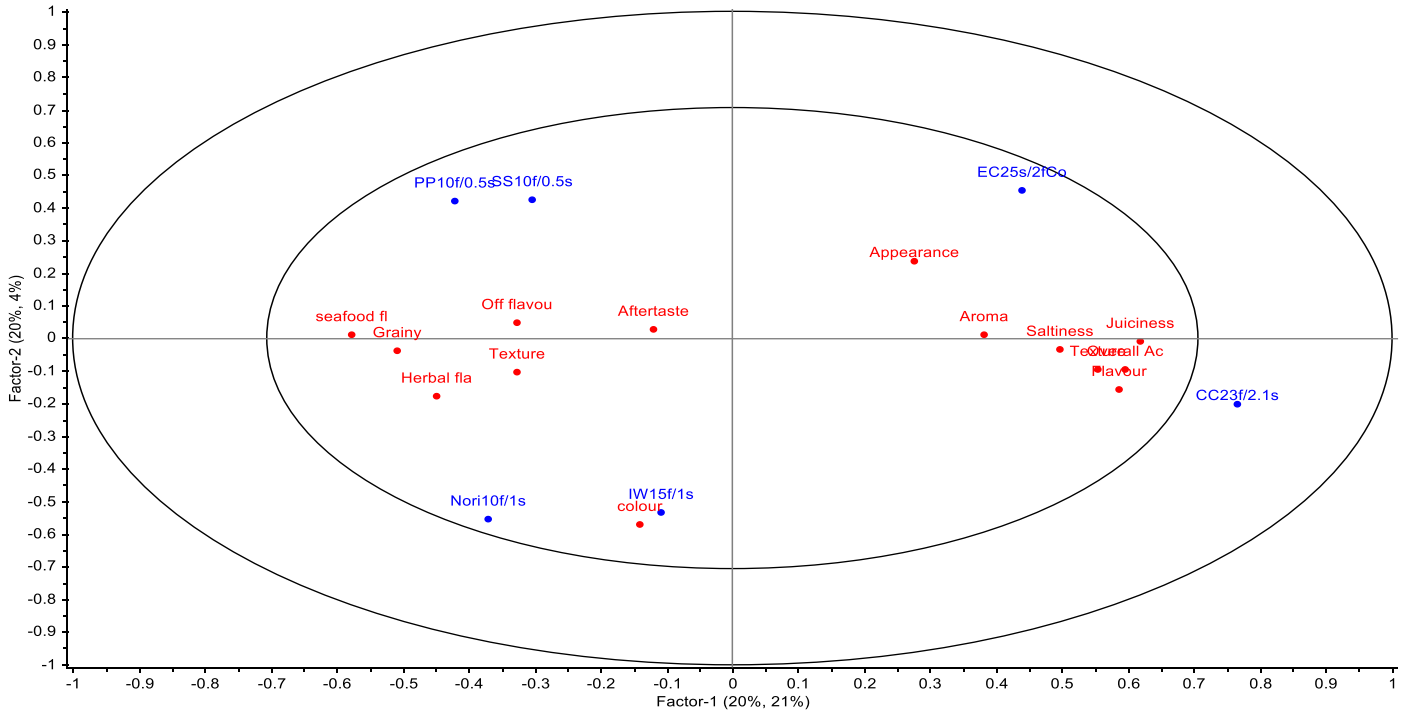


Figure 7.1. Partial least squares regression (PLSR) plot for the relationship between sausage samples (CC23f/2.1s- commercial control 23% fat 2.1% salt, EC25f/2s- Experimental control 25% fat 2% salt, PP10f/0.5s- dulse 10% fat 0.5% salt, Nori10f/1s- nori 10% fat 1% salt, SS10f/0.5s- sea spaghetti 10% fat 0.5% salt, IW15f/1s- Irish wakame 15% fat 1% salt (●)) and hedonic and intensity sensory variables (●)

Table 7.6. The significance of estimated regression coefficients (ANOVA values) for the relationship between sensory (hedonic and intensity) variables and sausage samples.

Samples	Hedonic					Intensity								
	Appearance	Flavour	Texture	Aroma	Overall Acceptability	Colour	Texture (Soft/Firm)	Juiciness (Dry/Moist)	Grainy	Saltiness	Seafood flavour	Herbal flavour	Off flavour	Aftertaste
CC23f/2.1s	0.000***	0.000***	0.000***	0.000***	0.000***	-0.197	-0.000***	0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.225
EC25f/2s	0.000***	0.000***	0.000***	0.005**	0.000***	-0.000***	-0.353	0.000***	-0.000***	-0.000***	-0.000***	-0.000***	-0.002**	-0.006**
PP10f/0.5s	-0.318	-0.000***	-0.000***	-0.108	-0.000***	0.000***	0.708	-0.000***	0.000***	-0.000***	0.000***	0.090	0.002**	0.123
Nori10f/1s	-0.000***	0.000***	0.000***	-0.001***	-0.000***	0.000***	0.000***	-0.000***	0.000***	-0.000***	0.000***	0.000***	0.003**	0.587
SS10f/0.5s	-0.473	0.000***	0.000***	-0.001***	-0.000***	0.000***	0.716	-0.000***	-0.006**	-0.000***	0.000***	0.288	0.002**	0.036*
IW15f/1s	-0.000***	-0.548	-0.799	-0.018*	-0.557	0.000***	0.000***	-0.036*	0.1087	-0.948	0.266	0.001**	0.494	0.512

Significance of regression coefficients; *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).

(-); indicates negative correlation.

CC23f/2.1s- commercial control 23% fat 2.1% salt, EC25f/2s- Experimental control 25% fat 2% salt, PP10f/0.5s- dulse 10% fat 0.5% salt, Nori10f/1s- nori 10% fat 1% salt, SS10f/0.5s- sea spaghetti 10% fat 0.5% salt, IW15f/1s- Irish wakame 15% fat 1% salt.

7.4. Conclusions

Hedonic sensory results obtained from the consumer (n=100) study revealed that the experimental control sausage sample was comparable to the commercial sample, and these samples were well-received. Reduced fat and salt sausages containing sea spaghetti (SS), Irish wakame (IW), dulse (PP) and nori were generally negatively perceived. Intensity sensory results highlighted positive associations of both commercial and experimental control samples with juiciness and saltiness, indicating desirable qualities. However, seaweed sausage samples exhibited negative associations with attributes such as texture, graininess, seafood flavour, herbal flavour, and off-flavours. Reduced-fat and -salt PP and SS sausage samples were situated close due to their fat and salt contents (10% fat and 0.5% salt) being the same, and consumers not perceiving much differences between these two, regardless of seaweed type. Nori10f/1s was the least liked and acceptable seaweed sausage. Overall, while seaweed incorporation in reduced fat and salt pork sausages holds promise for enhancing nutritional profiles, achieving optimal sensory acceptance using a wider audience remains a challenge. Further research is needed to improve the liking and acceptability of reduced-fat and -salt seaweed sausages, such as adding no-salt seasonings and flavours associated with saltiness in processed pork products, to contribute to the development of healthier and more palatable products that align with consumer preferences and dietary needs.

Chapter 8

Can odour induced salt enhancement (OISE) improve the sensory acceptability of reformulated pork sausages containing red Irish seaweed nori (*Porphyra umbilicalis*)?

Abstract

Odour-induced saltiness enhancement (OISE) is a method used to reduce the salt content in food. It is the use of aromas/flavours (stimuli) to compensate for salt reduction. OISE was used to attempt to improve the sensory acceptability of reformulated (reduced fat and salt) pork sausages containing red seaweed, nori (*Porhyra umbilicalis*). The sausage formulation contained 10% fat, 1% salt, 2.5% seaweed and 0.08% bacon stimuli. Physicochemical and sensory (optimized descriptive profiling (ODP)) properties were conducted. The addition of the red seaweed, nori, into reformulated sausages increased the ash content, reduced the L*a*b* values, and elevated the hardness and chewiness values. Reformulated sausage samples containing the seaweed nori (C2N and C2NB) were positively correlated ($P<0.001$) to appearance, seaweed aroma and grainy texture. Sausage sample C2B (reformulated sausage containing bacon stimuli) was positively associated with sausage ($P<0.05$) and bacon aroma ($P<0.001$), suggesting bacon stimuli may effectively enhance the overall flavour in reduced fat and salt pork sausages. Sausage samples C1 (control) and C2 (reformulated control) were positively correlated to juiciness and saltiness. Samples C1 and C2B were negatively associated ($P<0.05$) with hardness and C2N was positively correlated ($P<0.001$). Sample C2B was also positively correlated ($P<0.05$) to saltiness. To conclude, the bacon stimuli did not enhance the saltiness perception of reduced- fat and -salt sausage, containing nori seaweed (nori10f/1.0s).

8.1. Introduction

Processed meats such as pork sausages continue to be a prominent source of sodium in the Western diet (Fouladkhah *et al*., 2015). In pork sausages, sodium is found in a variety of forms, including sodium chloride (NaCl), monosodium glutamate (MSG), sodium diphosphates, sodium metabisulphite, and ascorbic acid sodium salt, with NaCl (salt) contributing the most. On the Irish market, fresh pork sausages typically contain between 1.7% and 2.4% salt. Excessive consumption of salt (sodium) has been linked to an increased risk of developing health conditions such as obesity, cardiovascular diseases and various cancers. However, the reduction of salt in pork sausages reduces the quality, functionality, microbial stability and sensory acceptability of the product.

Salt enhances the flavour of many foods up to tolerable levels, after which it diminishes the food's pleasantness (palatability) (Henney *et al*., 2010). Salt contributes more than simply a salty taste to the overall flavour of food. Salts' distinctive taste in foods is primarily brought about by the Na + cation in combination with the Cl − anion effect on receptor cells (Miller Jr, 1991; Murphy *et al*., 1981). The use of salt reduces water activity, which can result in a significant rise in the concentration of flavour components and an improvement in their volatility (Delahunty and Piggott, 1995; Hutton, 2002). Salt promotes the favourable sensory qualities of foods, including those that might otherwise be distasteful; it helps foods "taste" better (Henney *et al*., 2010). Additionally, salt improves the perception of meat flavour, which is a crucial aspect in the overall acceptance of meat products (Ruusunen and Puolanne, 2005). Research on a range of meals such as soups, rice, eggs, and potato chips, revealed that salt enhances the perception of product

thickness, enhances sweetness, masks metallic or chemical off-notes, and rounds the overall flavour while enhancing flavour intensity (Gillette, 1985). The non-salty sensory function of salt may be accentuated in foods that have reduced amounts of other favourable sensory qualities, like low-fat products (Henney *et al*., 2010). However, the use of ingredients such as seaweeds and flavour enhancing mechanisms such as odour induced salt enhancement (OISE) may improve the sensory attributes of reduced salt food products.

The red seaweed, nori (*Porphyra umbilicalis*) is also known as laver, purple laver, sloke, black butter, purple sea- vegetable and resemble strips of black plastic sacks attached to rocks when dried (Morrissey *et al*., 2001). Batches of nori seaweed used in this study have been assessed in chapter 3. This red seaweed is high in protein (32.03%) and hence, high amino acid (essential and non- essential) content (Chapter 3, section 3.3.3). Mabeau *et al*. (1992) observed that the high quantities of aspartic and glutamic amino acids in seaweeds are responsible for their distinctive flavour and taste (umami flavour). Furthermore, alanine, glycine, and glutamic amino acids were regarded as the primary components of seaweed flavour (Mišurcová *et al*., 2014). The flavour and taste of nori seaweed can be overpowering and overwhelming and thus disliked by consumers.

Odour-induced saltiness enhancement (OISE) is a method used to reduce the salt content in food. It is the use of aromas/flavours to compensate salt reduction in food. The multi-sensory interaction between aroma and taste could be used to make up the final salty taste perception of a healthy low-salt food (Shen *et al*., 2022). It has been demonstrated that taste can increase odour intensity and conversely, the perception of taste can be enhanced by odour (Salles, 2006). There are two ways of

receiving an odour: olfactory sensation (orthogonal sensory nerve conduction) and the retro nasal pathway (Shen *et al*., 2022). The integration across sensory modalities (odour/taste) is reflected in the orbitofrontal cortex, the dorsal mid-insula, and the perirhinal cortex (de Araujo *et al*., 2003; Seo *et al*., 2013; Seubert *et al*., 2015; Small *et al*., 2013).

Food aromas such soy sauce, sardine, beef, bacon, ham, goat cheese, chicken and tuna have been explored as OISE aromas in water solution (Batenburg and van der Velden, 2011; Chokumnoyporn *et al*., 2015; Djordjevic *et al*., 2004; Lawrence *et al*., 2009; Nasri *et al*., 2011, 2013; Onuma *et al*., 2018; Sinding *et al*., 2021). Also, comté cheese, sardine and butter aroma have been used in a food model like cheese as odour induced salt enhancers (Lawrence *et al*., 2011; Syarifuddin *et al*., 2016). To the best of our knowledge, OISE has not been explored in reformulated (reduced fat and reduced salt) meat products.

Reduced fat and salt nori sausage formulation (nori10f/1.0s) was selected from previous chapter (Chapter 7) as the least liked and acceptable seaweed formulation by consumers; hence, the need to improve its sensory acceptability. Therefore, the objective of this study was to determine if bacon stimuli can improve the sensory acceptability of reduced fat and salt nori sausage (nori10f/1.0s).

8.2. Materials and methods

8.2.1. Chemicals and reagents

Kjeltabs, sodium chloride (NaCl), hydrogen peroxide (H_2O_2), boric acid (H_3BO_3), hydrochloric acid (HCl) and sulphuric acid (H_2SO_4), were supplied by Sigma- Aldrich Ireland Ltd., Vale Road, Arklow, Wicklow, Ireland. Silver nitrate and Potassium chromate (K_2CrO_4) were purchased from Fischer Scientific, United Kingdom. Potassium dichromate ($K_2Cr_2O_7$) was supplied by BDH Limited Supplies, Poole, England. All solvents were of analytical grade.

8.2.2. Raw materials

Red seaweed *Porphyra umbilicalis* (Nori) was purchased from Wild Irish Seaweed, Co. Clare, Ireland. According to the supplier's specifications, seaweed was air-dried, subsequently dehumidified, and milled to 1mm in size. Seaweed was hand-harvested off the coast of Co. Clare, Ireland, and were 100% naturally grown, sustainably harvested and organically certified.

Pork oyster muscle and backfat were supplied by Ballyburden Meat Processors (Cork, Ireland). Food-grade salt was obtained from British salt Ltd. (Cheshire, UK). Reduced salt sausage seasoning containing rusk [wheat flour (calcium carbonate, Iron, Niacin, Thiamin), salt], sugar, dextrose, stabiliser (E450, E452), flavour enhancer (E621), preservative (E223), antioxidant (E301), natural flavourings, anticaking agent (E551), rapeseed oil, hydrolysed vegetable protein (soya, maise)) was obtained from Redbrook Ingredient Services (Mulhuddart, Dublin 15, Ireland). Rusk [Wheat flour (wheat flour, calcium carbonate, Iron, Niacin, Thiamin), salt, raising agent E503] and

collagen casing were purchased from Viscofan UK Ltd. (Sevenoaks, Kent, UK). Bacon and boiled ham flavours (stimuli) were purchased from Silesia, Neuss, Germany.

8.2.3. Stimuli selection

A preliminary test was done to select the appropriate flavour and concentration, as a sensory selection of odours associated with saltiness would be time consuming. The stimuli selected had previously been studied by Lawrence *et al*. (2009). Bacon and ham from Silesia, Neuss, Germany were selected were selected based off this study and their similarity to pork sausages. These two stimuli were presented to a group of 5 assessors, who assessed for odour and taste intensity at four concentrations: 0.02%, 0.04%, 0.06% and 0.08% (diluted in water) and a control (water only). According to the manufacturer, 0.08% is the maximum level to be added to food products. The assessors confirmed these stimuli had no taste and colour, and also received the odour through the olfactory and retro nasal pathways. All 5 assessors preferred the bacon flavour over the boiled ham flavour. They also selected the bacon flavour at a concentration of 0.08% as the most suitable. Lawrence *et al*. (2009) rated bacon as a top OISE stimuli in water solution (0.025%) and was also considered as one of the most saltiness-associated food names.

8.2.4. Sausage manufacture

Fresh pork sausages (4.3 kg batches) (Control 1 (C1)) were formulated containing 41.1% pork oyster muscle, 25% pork back fat (f), 18.5% water (50:50, water: ice), 11.4% rusk and 2.0% seasoning and 2% salt (s) (0.39% salt from the sausage seasoning, 0.14% from the rusk plus 1.5% salt added as an ingredient into the

formulation), and control 2 (C2) was formulated containing 57.1% pork oyster muscle, 10% pork back fat (f), 18.5% water (50:50, water: ice), 11.4% rusk and 2.0% seasoning and 1.0% salt (s) (0.39% salt from the sausage seasoning, 0.14% from the rusk plus 0.5% salt added as an ingredient into the formulation). Additional batches of pork sausages (n=3) were manufactured (Table 8.1). Reduced fat and salt nori sausage formulation (nori10f/1.0s) was selected from previous chapter (chapter 6) as the least liked and acceptable seaweed formulation by consumers (n=100).

Sausages were manufactured by mincing the pork meat and backfat through a 4mm perforated plate (Talsabell SA., Valencia, Spain). Pork oyster, half of the water, salt, and the seasoning was mixed for 60 seconds at high-speed using a bowl chopper (Seydleman Bowl chopper, Burgstallstraße, Germany). The backfat and various red seaweeds were added and mixed at high speed for 30 seconds. The other half of the water and bacon stimuli were added, and the batter was mixed for 30 seconds at high speed. Rusk was added and mixed at low speed for 30 seconds. Sausage batter was stuffed using a piston-type sausage filler (Mainca UK Ltd., Berkshire, UK) into 21mm diameter collagen casings and hand-linked (~ 10 cm in length).

Table 8.1. Experimental control and reduced fat and salt seaweed sausages formulations (%).

Treatment	Pork oyster	Pork back fat	water	Rusk	Seasoning	Salt	Seaweed	Bacon stimuli
C1	41.10	25.0	18.5	11.4	2.0	2.0	0	0
C2	57.10	10.0	18.5	11.4	2.0	1.0	0	0
C2N	54.60	10.0	18.5	11.4	2.0	1.0	2.5	0
C2B	57.02	10.0	18.5	11.4	2.0	1.0	0	0.08
C2NB	54.52	10.0	18.5	11.4	2.0	1.0	2.5	0.08

C1- control, C2- reduced fat and salt control, C2N- C2+ nori seaweed, C2B- C2 + bacon stimuli, C2NB- C2 + nori seaweed + bacon stimuli

8.2.5. Physicochemical properties

8.2.5.1. Proximate composition

Protein (Kjeldahl) and ash (muffle furnace at 550 ^{o}C) contents were analysed by AOAC Methods 954.01 and 942.05, respectively (AOAC, 1999). Moisture and fat content of sausages were determined using a SMART Trac system (CEM GmbH, Kamp- Lintfort, Germany). Results were expressed as g/100g of sample.

8.2.5.2. Instrumental colour analysis

The colour of fresh sausage samples was measured according to the CIE L*a*b* colour system. The samples were allowed to equilibrate at room temperature for 30 minutes before measuring. The surface colour was measured using a Minolta chromameter (CR400, Minolta Camera Co.Ltd, Osaka, Japan) with an 11mm-diameter aperture and D65 illuminant. The chromameter was calibrated using a white tile (L=97.79, a=-0.11, b= 2.69).

8.2.5.3. Texture profile analysis

The texture profile analysis (TPA) of sausage samples was carried out using a texture analyser TA-XF10S1.5i (Stable Micro System, Surrey, England) based on a method described by Bourne (1978). Cooked sausage samples were allowed to equilibrate at room temperature for 30 minutes before measuring. The samples were cut into cylindrical slices (10 mm thickness), and four pieces were measured per sausage sample. The samples were subjected to a two-cycle compression using a 30 kg load cell. All samples were compressed to 40% of their original height using a cylindrical probe (SMP/35 compression plate) at a cross-head speed of 1.5 mm/s. Determining factors include; hardness (N), the maximum force required for the first compression; springiness (mm), distance the sample recovers after initial compression; cohesiveness (dimensionless), the ratio of positive force area during the second compression and chewiness (N*mm), the product of gumminess and springiness.

8.2.5.4. Water holding capacity

The water holding capacity (WHC) was measured using the method described by Lianji and Chen (1989). Fresh sausage samples (10g) were weighed into glass jars and heated in a water bath for 10 min at 90 ^{o}C. After heating, the samples were removed, wrapped in cheesecloth, and placed in 30ml centrifuge tubes lined with cotton wool at the bottom of each tube. The samples were centrifuged (Avanti J-E centrifuge, Beckman Coulter, California, USA) at 13,300g for 10 min at 4°C. After centrifugation, the cheesecloth was removed, and samples were reweighed. WHC was calculated using the following calculation:

$$\% \text{ Water holding capacity} = 1 - \frac{(B-A)}{(B \times M)} \times 100$$

Where B is the sample's weight before heating, A is the weight of the sample after heating and centrifuging, and M is the % moisture of the sample.

8.2.5.5. Cook loss

Sausage weight was recorded before and after cooking. Sausages were cooked in a Zanussi oven at 180 ^{o}C for 22 min until an internal temperature of 72 ^{o}C was reached. Cook loss was calculated using the following equation:

$$\% \text{ Cook loss } = \frac{\text{Raw weight-cooked weight}}{\text{Raw weight}} \times 100$$

8.2.6. Sensory analysis (Optimised Descriptive Profiling)

Optimized descriptive profiling (ODP) is a rapid method for obtaining sensory descriptions utilizing semi-trained judges that has the potential to quantitatively evaluate sensory attributes (da Silva *et al*., 2012). ODP was carried out in the panel booths of the sensory science laboratory, at University College Cork. A panel of assessors who were regular consumers of sausages were recruited, trained and participated in ODP according to the method of da Silva *et al*. (2012). The study took place over four days, one hour session per day. On day one, the score sheet was presented and demonstrated to assessors, different salt concentrations (0.5%. 1%, 1.5% and 2%) and bacon stimuli diluted in water at concentrations 0.02%, 0.04%, 0.06% and 0.08% were also presented to assessors for evaluation, training and familiarisation. panellists (n= 21) were selected based on this training. On the second and third days, specific protocols were defined, sausages samples with different salt and nori seaweed concentrations were presented to assessors and discussed to develop and agree on attributes. Familiarisation with the scales was also done. On

the fourth day, the samples were evaluated. Crackers and water were used as taste palate cleansers, and coffee to cleanse olfactory palate.

The consensus list of descriptors (Table 8.2) was measured on a 10 cm continuous line scale. The terms 'none' and 'extreme' were used on the 0 cm and 10 cm anchor points on the scale respectively (unless stated otherwise in Table 8.2). All samples were scored on the same scale for each descriptor. Samples were served coded in randomised order and presented simultaneously to assessors (Stone *et al*., 2020). All subjects gave their informed consent for inclusion before they participated in the sensory study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by university college cork social Research and Ethics Committee (Log 2021-188).

Table 8.2. Consensus list of sensory descriptors and definitions.

Attribute	Anchor points on scale	Definition
Appearance	Light - dark	Assessment of internal colour. Degree of lightness and darkness
Sausage aroma	None - extreme	Aroma associated with typical Irish pork sausages
Bacon aroma	None - extreme	Aroma associated with smoky bacon
Seaweed aroma	None - extreme	Aroma associated with seaweed
Hardness	Soft - hard	Degree of hardness
Juiciness	Dry - moist	Degree of moisture
Saltiness	None - extreme	Degree of saltiness
Grainy	None - extreme	Degree of grittiness
Umami flavour	None - extreme	Savoury flavour- like in broths, stock cubes and cooked meats
Spiciness	None - extreme	Presence of spices; not hot flavour

Off flavour	None - extreme	Flavour not characteristic to pork sausages and rancid flavour
After taste (bitter/ acidic)	None - extreme	Lingering taste after swallowing

8.2.7. Statistical analysis

Physiochemical analyses were carried out on three independent trials, on duplicate samples per trial and three readings were taken per sample. Data were presented in triplicate (average per trial). Statistical analysis was carried out using the IBM SPSS statistics 25 for windows (SPSS, Chicago, IL, USA) software package. One-way ANOVA was used to compare means and Tukeys post-hoc test was used to adjust for multiple comparisons between treatment means at a 5% significance level.

Raw data obtained from sensory analysis (ODP) was coded into Microsoft excel. The relationship between the set of samples and the set of sensory variables was determined by ANOVA- partial least squares (APLS) regression using Unscrambler software (Unscrambler 10.3 CAMO software ASA, Trondheim, Norway). The X-matrix was designed as 0/1 variables for sample treatments and the Y-matrix contained sensory variables. Data were normalised during pre-processing, by taking the logarithm to achieve uniform precision over the whole range of variation. Regression coefficients were analysed by Jack-knifing, which is based on cross-validation and stability plots (Martens and Martens, 2000). Principal component (PC) 1 versus PC 2 are presented; other PC's did not yield additional information. Statistical significance for the relationships analysed were defined as $P<0.05$-0.01 (significant), $P<0.01$-0.001 (highly significant) and $P<0.001$ (extremely significant).

8.3. Results and Discussion

8.3.1. Physicochemical properties of sausage formulations

Table 8.3 shows the physicochemical properties of reduced salt and fat pork sausages containing nori seaweed and bacon stimuli. Sausage treatment C1 had lower ($P<0.05$) protein and moisture contents, and higher ($P<0.05$) fat content than other treatments. Like in chapter 6, this is as a result of the lower pork oyster and higher pork backfat in the control (C1) formulation. C1 also had the highest ($P<0.05$) ash content, followed by nori containing sausages (C2N and C2NB), then C2 and C2B sausage treatments. The ash content is total amount of inorganic minerals, like sodium and potassium present in a food sample. C1 had a high sodium (salt) content, hence it being the sample with the highest ash content. Minerals present in nori seaweed (chapter 3, section 3.3.4) contributed to the total ash content of the sausages (C2N and C2NB).

C2N had significantly higher WHC than C2 sausage treatment. Generally, seaweed sausages (C2N and C2NB) had elevated WHC values when compared to other sausage treatments. The lightness (L*) value of C1 was the highest ($P<0.05$), and C2N and C2NB had the lowest ($P<0.05$) values. Sausage treatments, C2N and C2NB, had significantly lower a* (redness) and b* (yellowness) values. This reduced L*a*b* values is as a result of the reported high levels of carotenoids (β-carotene, lutein) and pigments (chlorophyll a, pheophytin a) in nori seaweed (Ferraces-Casais *et al*., 2012). Widati *et al*. (2021) also noted that the addition of red seaweed, *Eucheuma cottonii*, contributed positively to the ash content and reduced L*a*b* values of cooked Indonesian-style beef meatball. C2N and C2NB also had increased ($P<0.05$) hardness and chewiness. Carrageenan, a polysaccharide present in nori seaweed has been

noted to increase hardness and chewiness because of the gel-like nature in reduced fat pork meat products, which are comparable to that of high fat pork patties (Giese, 1996; Kumar and Sharma, 2004).

Physicochemical results in this chapter are consistent with reduced fat and salt nori sausages (nori10f/ 1.0s) in Chapter 6. The main purpose of using bacon stimuli as a factor in the product optimisation is to enhance the sensory acceptability and this confirmatory study is underway.

8.3.2. Sensory analysis (Optimised Descriptive Profiling (ODP)

The relationship between sensory terms and sausage samples is presented in the APLSR plot in Figure 8.1 with the corresponding significance of estimated regression coefficients in Table 8.4. Sausage samples C1, C2 and C2B were negatively correlated ($P<0.001$) to appearance, while sausage samples containing the seaweed nori (C2N and C2NB) were positively correlated ($P<0.001$) to appearance. The incorporation of the red seaweed, nori, impacted the colour of the sausages, making it darker. Red seaweeds contain the pigment, phycobilin (phycoerythrin and phycocyanin), which is known to cause darker colour changes in sausages (Munsu *et al*., 2021).

With regards to the sausage aroma descriptor, sausage samples C2, C2N and C2NB were negatively associated and C2B was positively associated ($P<0.05$). These results suggest that the aroma exerted by nori seaweed overpowered the bacon stimuli aroma in sausage sample C2NB. Also, the bacon stimuli present in C2B can be associated with sausage aroma as both bacon and pork sausages can be classified as processed pork products. Samples C1 and C2B were positively correlated to bacon

aroma while C2N was negatively correlated (P<0.001). Sample C1 contained high fat (25%) and salt (2%) contents and hence, may be associated with bacon aroma.

Samples without the seaweed nori (C1, C2 and C2B), were negatively associated with seaweed aroma and grainy texture, and samples containing the seaweed nori (C2N and C2NB), were positively associated (P<0.001) with seaweed aroma and grainy texture. Volatile compounds like 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 present in nori and potent odorants in seafood contributing to the aroma composition of seaweeds (Höckelmann and Jüttner, 2005; López-Pérez *et al*., 2017).

The relationship between control sample C1 and descriptor juiciness was positively correlated (P<0.001). Sausage sample C2 was also positively correlated (P<0.05) to juiciness. Samples C1 and C2B were negatively correlated (P<0.05) to hardness and C2N was positively correlated (P<0.001) to hardness. Samples C1 and C2 were positively correlated (P<0.001) to saltiness. Sausage sample C2B was also positively associated (P<0.05) with saltiness. Control sample C1 was perceived as moist, soft and salty as this contained high fat (25%) and salt (2%) contents. However, reduced fat and salt control (C2) was also perceived as juicy and salty. Fat is known to aid in the development of texture and mouth feel, as well as the overall sensation of lubricity in foods (Giese, 1996). Also, salt solubilizes myofibrillar proteins, and an increase in salt results to more solubilisation, which increases water holding capacity and consumer perception of juiciness. Hence, the higher the fat and salt contents present, the more juiciness is perceived (Tobin *et al*., 2013).

Panellists perceived sausage sample C2B as soft and salty, regardless of it containing low fat and salt, this suggests bacon stimuli may have a positive effect on the texture (hardness) and salty perception of reduced fat and salt sausages. Also, carrageenan, a polysaccharide present in red seaweeds has been reported to increase the hardness of reduced fat pork patties (Kumar and Sharma, 2004); hence, the positive correlation between sausage sample C2N and hardness. Furthermore, reduced fat and salt control sausage sample C2 was negatively associated ($P<0.05$) with umami flavour and aftertaste.

Table 8.3. Physicochemical properties of reduced salt and fat pork sausages containing nori seaweed and bacon stimuli.

	Treatment				
	C1	C2	C2N	C2B	C2NB
Proximate composition					
Protein	12.75±0.08^{a}	14.43±0.09^{b}	14.58±0.23^{b}	14.74±0.18^{b}	14.70±0.21^{b}
Fat	20.26±0.53^{a}	9.19±0.44^{b}	9.27±0.32^{b}	9.47±0.11^{b}	9.59±0.29^{b}
Ash	2.79±0.16^{a}	1.81±0.03^{b}	2.17±0.04^{c}	1.79±0.03^{b}	2.11±0.02^{c}
Moisture	51.94±0.58^{a}	59.24±0.18^{bc}	60.42±0.97^{c}	59.63±0.29^{c}	57.33±0.89^{b}
Technological properties					
WHC	61.47±1.80^{ab}	60.67±2.20^{a}	69.20±4.63^{b}	62.15±2.40^{ab}	67.48±2.47^{ab}
Cook loss	22.52±0.09^{a}	24.83±1.73^{a}	23.88±2.84^{a}	25.92±1.14^{a}	23.18±0.68^{a}
Surface colour					
L*	71.57±0.83^{a}	65.61±0.59^{b}	48.84±0.72^{c}	66.06±0.20^{b}	49.65±1.30^{c}
a*	7.24±0.27^{a}	7.72±0.57^{a}	5.36±0.29^{b}	8.00±0.05^{a}	5.09±0.66^{b}
b*	14.43±0.34^{a}	14.85±0.48^{a}	7.10±0.49^{b}	14.58±0.39^{a}	6.57±0.86^{b}
TPA					
Hardness	40.94±2.53^{ab}	38.09±4.03^{a}	41.05±2.36^{ab}	38.10±2.09^{a}	45.22±0.65^{b}
Springiness	0.902±0.03^{a}	0.929±0.01^{a}	0.925±0.01^{a}	0.930±0.03^{a}	0.911±0.02^{a}
Cohesiveness	0.653±0.01^{a}	0.658±0.02^{a}	0.703±0.03^{a}	0.664±0.02^{a}	0.700±0.01^{a}
Chewiness	23.81±1.94^{a}	28.72±1.94^{ab}	32.11±0.08^{b}	28.98±0.97^{ab}	34.34±4.57^{b}

^{abc} For each seaweed, within each parameter, mean values (±standard deviation) in the same column bearing different superscripts are significantly different ($P<0.05$).

C1- control 1, C2- reduced fat and salt control, C2N- C2+ nori seaweed, C2B- C2 + bacon stimuli, C2NB- C2 + nori seaweed + bacon stimuli.

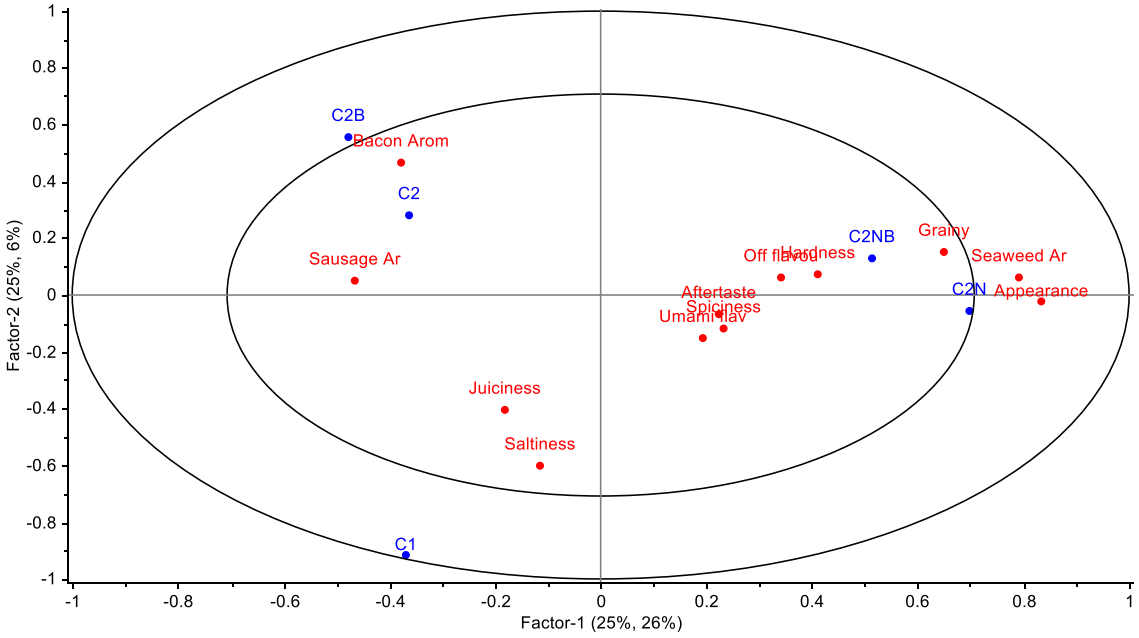


Figure 8.1. Partial least squares regression (PLSR) plot for the relationship between sausage samples (C1- control 1, C2 reduced fat and salt control, C2N- C2+ nori seaweed, C2B- C2 + bacon stimuli, C2NB- C2 + nori seaweed + bacon stimuli ( )) and sensory (ODP) variables ( )

Table 8.4. The significance of estimated regression coefficients (ANOVA values) for the relationship between sensory (ODP) variables and sausage samples.

Treatment	Appearance	Sausage Aroma	Bacon Aroma	Seaweed Aroma	Hardness	Juiciness	Grainy	Saltiness	Umami flavour	Spiciness	Off flavour	Aftertaste
C1	-0.0000***	0.2852	0.0031**	-0.0000***	-0.0323*	0.0000***	-0.0001***	0.0000***	0.4702	0.8028	0.0630	0.9501
C2	-0.0000***	-0.0308*	0.7565	-0.0000***	0.7669	0.0181*	-0.0086**	0.0009***	-0.0489*	0.1289	0.2998	-0.0252*
C2N	0.0000***	-0.0003***	-0.0000***	0.0000***	0.0009***	0.0617	0.0000***	0.3647	0.1929	0.1948	0.0847	0.2499
C2B	-0.0000***	0.0392*	0.0000***	-0.0000***	-0.0278*	0.8385	-0.0393*	0.0445*	0.3551	0.0915	0.1420	0.3977
C2NB	0.0000***	-0.0154*	0.9519	0.0000***	0.2238	0.9327	0.0002***	0.4433	0.4098	0.3030	0.0820	0.2506

Significance of regression coefficients; *P<0.05-0.01 (significant), **P<0.01-0.001(highly significant), ***P<0.001(extremely significant).
(-); indicates negative correlation.
C1- control 1, C2- reduced fat and salt control, C2N- C2+ nori seaweed, C2B- C2 + bacon stimuli, C2NB- C2 + nori seaweed + bacon stimuli

8.4. Conclusions

There are factors that impact odour induced salt enhancement (OISE), such as types and concentration of odorants (stimuli), salt concentration or salty-taste intensity before adding an odour and other compositions in food matrix (Thanarungroj and Kongpensook, 2021). To add to this, other ingredients such as seaweeds in the food matrix may also impact the effectiveness of selected stimuli. Sensory analysis through Optimised Descriptive profiling (ODP) provided insights into the sensory attributes of the sausage samples. Sausages containing nori seaweed (C2N and C2NB) exhibited darker appearance, positively correlated with seaweed aroma and grainy texture, and negatively associated with bacon aroma. The incorporation of nori seaweed impacted sausage aroma, overpowering the bacon stimuli aroma in sample C2NB. Control sample C1 was perceived as moist, soft, and salty due to its high fat and salt contents. Reduced fat and salt control C2 also exhibited juiciness and saltiness perception. The bacon stimuli in C2B positively affected the salty perception, suggesting a potential enhancement of sensory attributes by the inclusion of bacon stimuli.

The findings underscore the co plex interplay between ingredient composition, sensory attributes and consumer perception in the development of reduced fat and salt pork sausages. The incorporation of nori seaweed and bacon stimuli presents opportunities to manipulate sensory characteristics, although further optimisation is necessary to achieve desired flavour profiles and overall acceptability. Future research endeavours should focus on exploring other salt related stimuli at higher concentrations and consumer preference studies to drive product innovation and market success.

CHAPTER 9.

General discussion, conclusions and future work

9.1. General discussion

The excessive- or over-consumption of processed meat products, such as high fat and high salt containing sausages can have negative impacts on human health and concerns over the consumption of saturated fat and salt in general food processing has prompted much research into the reformulation and development of healthier foods, including processed meat products. Sausages are one of many processed food categories which can be significant contributors of saturated fat and salt (sodium) in the Irish diet, depending on specific product formulation. The relationship between dietary high fat and salt intake and chronic diseases such as cancer, obesity and cardiovascular disease has been well established. As a result, regulatory agencies such as the FSAI and WHO have set targets for saturated fat and salt reduction in processed foods, including meat products, and recommended daily intake (FSAI, 2022; WHO, 2012). Hence, the aim of this thesis was to examine the effect of Irish brown (*Himanthalia elongata*- sea spaghetti and *Alaria esculenta*- Irish wakame) and red (*Palmaria palmata*- dulse, *Porphyra umbilicalis*- nori) seaweed species in sausages- characterisation of these seaweed species, fat and salt reduction reformulations, and sensory acceptability studies.

In the initial part of this thesis (Chapter 1), a review of the literature was conducted to highlight the positive benefits and negative impacts of consuming processed meat products, mainly focusing on excess fat and salt consumption. The health impacts, role and reduction strategies of saturated fat and salt were highlighted. Studies on the use of fat replacers such as gums, dietary fibre and starches in meat products have been conducted, and these compounds can form a strong matrix and a high water-holding capacity, which leads to an increase in yield and an improvement in

the texture of meat products. Potassium chloride (KCl), magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) are a few examples of salt replacers which have been explored in meat products. However, using KCl has negative consequences on the product's flavour (produces metallic flavour) and texture. Furthermore, few studies measured the physicochemical and sensory properties of simultaneously reduced-fat and -salt in processed meat products such as pork sausages, white and black puddings, frankfurters and beef patties, with and without replacers. Replacers such as brown and red seaweeds may be adequate to improve the physicochemical, sensory and shelf life properties of reduced fat and salt sausages.

The use of seaweeds as fat and salt replacers was highlighted in this review. Seaweeds contain polysaccharides (dietary fibre) that may enhance sausage fat-like texture and flavour. Minerals such as sodium and potassium are present in brown and red seaweeds in high amounts, and these minerals can present a salt flavour and taste in meat products. While they possess these properties to act as fat and salt replacers, they also contain other nutritious components such as vitamins, amino acids and bioactive compounds that could improve the nutritional component of meat products. The use of brown seaweed wakame was studied as a fat and salt replacer in white puddings and was perceived as salty and spicy by consumers. From examination of the literature, no work had been identified on the use of Irish brown (*Himanthalia elongata*- sea spaghetti and *Alaria esculenta*- Irish wakame) and red (*Palmaria palmata*- dulse, *Porphyra umbilicalis*- nori) seaweeds on the simultaneous reduction of fat and salt in meat products, i.e. pork breakfast sausages; hence their selection. These brown and red seaweeds were also selected due to their availability, possible positive contribution to the appearance and sensory acceptability of pork

sausages. Green seaweeds were not selected due to their excessive chlorophyll which may negatively impact the sensory acceptability of these products, particularly the appearance.

OISE is another strategy used to reduce salt and was extensively reviewed (Chapter 1). OISE uses aromas/flavours to compensate for salt reduction in foods by delivering a salty perception. As promising as this strategy is, it has only been explored in water samples and cheese models. Work had yet to be carried out on using this strategy for salt reduction in meat products. Thus, scrutiny of the scientific literature highlighted the need to measure the impact of a simultaneous replacement of fat and salt in pork sausages, using brown and red Irish seaweeds to determine the levels of seaweeds, fat and salt needed for sensory acceptance. Additionally, we wanted to determine if OISE can improve the sensory acceptability of seaweed reformulated pork sausages. Consumer knowledge and attitudes towards seaweed and seaweed food products were reviewed in Chapter 1. Surveys on this topic have been conducted in countries such as Spain, France and Australia. From evaluation of the literature, data had yet to be collected on the knowledge and attitudes of Irish consumers on the intake of seaweed and seaweed food products. Thus, this became the main objective of the first experimental study carried out as part of this work (Chapter 2). Therefore, from extensive review of the scientific literature, it was clear that there was requirement for research in this area, based on knowledge gaps identified, and therefore, a scientific plan was drafted to investigate the use of commercially available and edible Irish soured seaweeds as ingredients in processed meats, the model chosen being breakfast pork sausages.

Experimental research commenced with a survey which focused on consumer knowledge and attitudes towards seaweed and seaweed food products (Chapter 2). This chapter played a crucial role in comprehending the level of consumer acceptance towards seaweed and the existing seaweed food products on the market. In general, Irish consumers demonstrated a favourable reception towards seaweeds and seaweed-based food items. Furthermore, individuals belonging to the younger demographic (21-29 years old) indicated a higher level of confidence in unfamiliar food items and displayed reduced reservations regarding the consumption of new food products. The findings of this survey indicate that Irish consumers exhibit positive views and possess a strong understanding of seaweeds, seaweed products, and the health benefits they offer and the target consumers of seaweed food products are most likely going to be a younger demographic (21-29 years old). This promising opportunity for the utilisation, commercialization, and acceptance of seaweed-based products, such as breakfast sausages, within the Irish market should be explored by food producers.

Chapter 3 focused on the utilisation of edible and commercially available Irish brown (*Himanthalia elongata*- sea spaghetti and *Alaria esculenta*- Irish wakame) and red (*Palmaria palmata*- dulse, *Porphyra umbilicalis*- nori) seaweed species in pork sausages. These seaweeds represent potential nutritional sources of protein, amino acids, dietary fibre, minerals and health–promoting bioactive antioxidant compounds in the human diet. Seaweed species in this study contained low fat levels, thereby reducing their caloric content when ingested alone or as a component of a food product. Seaweeds had thermal stability at a high temperatures. This study

demonstrates the potential applicability of these seaweeds as ingredients in the production of healthier and novel processed food products.

In chapter 4, the incorporation of edible and commercially available Irish brown (*Himanthalia elongata*- sea spaghetti (SS) and *Alaria esculenta*- Irish wakame (IW)) and red (*Palmaria palmata*- dulse (PP), *Porphyra umbilicalis*- nori) seaweed species into pork sausages were examined to identify the maximum acceptable inclusion levels. The incorporation of seaweeds (nori, SS, and IW) at a 5% concentration and PP at 2.5% and 5% in pork sausages had a detrimental effect on the colour, instrumental textural attributes, and sensory characteristics. Irrespective of the influence exerted by these seaweeds on the colour of the sausage samples, the panellists' preference for their appearance was similar to that of the control group. The sensory findings revealed that consumers exhibited a preference for sausages with a lesser proportion of seaweed. Furthermore, the preference for seaweed sausages was ranked in the following order: nori > SS > IW > PP. The sensory findings also indicated that the inclusion level of PP in pork sausages should not exceed 1%, while the other seaweed species tested showed a maximum acceptable inclusion level of 2.5%. These inclusion levels did not have any detrimental effects on the quality, functioning, and sensory acceptance of the sausages. From this study, it is apparent that seaweeds can be acceptable in a range of food products such as sausages, thereby developing novel and sustainable foods. However, the maximum acceptable level for each seaweed specie needs to be established for each food product.

In the following chapter (chapter 5), the investigation of the use of brown seaweed species (*Himanthalia elongata*- sea spaghetti (SS) and *Alaria esculenta*- Irish wakame

(IW)) as ingredients in fat and salt reduced sausages, at the predetermined acceptable inclusion levels (2.5%) established in the previous chapter (chapter 4). The panellists expressed a positive reception towards SS treatments, but IW treatments demonstrated comparatively lower sensory scores. This follows the trend from Chapter 4, where panellists preferred SS sausages to IW sausages. The sausage formulations chosen were SS10f/0.5s, 10% fat and 0.5% salt, and IW15f/1.0s, 15% fat and 1% salt. The results obtained from lipid oxidation and microbiological analysis demonstrated that the MAP70/30 (70% N_2: 30% CO_2) and vacuum packaging methods had the best effectiveness, respectively. This study emphasises the appropriateness of SS and IW as ingredients to improve the nutritional value of pork sausages by reducing fat and salt by 40-60% and 50-75% respectively. This surpasses the target of reducing fat and salt by 10% by 2025, as set by FSAI (2022).

The study conducted in Chapter 6 investigated the application of red (*Palmaria palmata*-dulse (PP) and *Porphyra umbilicalis*- nori) seaweed species as ingredients in fat and salt reduced sausages. The inclusion levels of 1% PP and 2.5% nori were determined based on the specified acceptable thresholds established in the preceding chapter (chapter 4). The results of the surface colour examination indicated that the seaweed sausages exhibited a usually darker colour. The texture profile analysis (TPA) revealed that the seaweed sausages exhibited texture properties that were similar to those of the control group. The sensory analysis results indicated that the seaweed sausages were positively received by the panellists, with PP sausages exhibiting higher levels of acceptance compared to those made with nori sausages. These sensory findings differ significantly from the ones obtained in chapter 4, as nori was favoured over PP. This implies that modifying the

formulation, specifically by reducing fat and salt content, leads to changes in consumer preference. The sausage formulations selected were PP10f/0.5s with 10% fat and 0.5% salt, and nori10f/1.0s with 10% fat and 1.0% salt. The findings from the analysis of shelf-life stability indicated that the packing condition MAP70/30 (70% N2: 30% CO2) had the highest efficacy in terms of lipid oxidation and microbiological safety. The present study highlights the suitability of PP and nori as constituents for enhancing the nutritional value of pork sausages by a reduction in fat and salt content by 60% and 50-75%, respectively. This exceeds the objective of achieving a 10% reduction in fat and salt by 2025, as established by FSAI (2022).

The sensory analysis findings presented in chapters 5 and 6 indicate that seaweeds with a fat content of 10% and a salt content of 0.5% (SS10f/0.5s and PP10f/0.5s) exhibited the highest level of favourability. It is likely that sea spaghetti (SS) and dulse (PP) possess fewer unpleasant flavours compared to Irish wakame and nori, when incorporated into sausages. The optimal packaging for prolonging the shelf life of these reduced fat and salt seaweed sausages, which effectively prevents lipid oxidation and microbiological growth, is MAP70/30 (70% N2: 30% CO2).

Chapter 7 presents the hedonic sensory findings from a study involving 100 consumers. The results indicated that both the experimental control and commercial sausage samples were deemed acceptable and comparable by consumers. This finding serves to validate the market standard of the experimental control employed in this thesis. The sausages made with reduced fat and salt, which included sea spaghetti (SS), Irish wakame (IW), dulse (PP), and nori, were largely viewed unfavourably. This study's findings exhibit notable disparities compared to the sensory outcomes reported in chapters 5 and 6, whereby a more limited sample size

of 25 panellists was employed. The sensory results of PP and SS sausage samples were comparable since they had the same fat and salt contents (10% fat and 0.5% salt), and consumers did not perceive significant changes between the two, regardless of the type of seaweed used. The seaweed sausage Nori10f/1s received the lowest appeal and acceptance. While seaweed incorporation in reduced fat and salt pork sausages shows potential for increasing nutritional profiles, getting ideal sensory acceptance employing a wider audience remains a challenge.

Chapter 8 investigated the application of the Odour Induced Saltiness Enhancement (OISE) method, which involves using bacon stimuli, to enhance the flavour and acceptability of the seaweed formulation that was least preferred and accepted by consumers (nori10f/1.0s) in the previous chapter (Chapter 7). This was done using Optimised Descriptive Profiling (ODP). The sausages that incorporated nori seaweed (C2N and C2NB) displayed a darker visual appearance. These samples were also positively correlated with seaweed aroma and a grainy texture. Conversely, there was a negative association observed with bacon aroma. The addition of nori seaweed had a significant effect on sausage aroma surpassing the aroma of bacon stimulus in sample C2NB. Control sample C1 was viewed as possessing moisture, softness, and saltiness, which can be attributed to its elevated levels of fat and salt. The incorporation of bacon stimuli in C2B had a positive impact on the perception of saltiness, indicating a potential improvement in sensory characteristics. The study elucidated that the incorporation of bacon stimulus does not enhance the taste and desirability of sausage formulation, nori10f/1.0s. Nevertheless, it can be employed in the reduction of salt by 60% in pork sausages.

9.2. Conclusions

The general conclusions from this thesis are as follows:

- Irish consumers (87.2%) trust newly developed foods, and 89.3% are not intimidated by eating new foods as they emerge on the market (Chapter 2).
- Most (73.6%) Irish consumers have consumed seaweed or a seaweed-containing products (Chapter 2).
- Brown (sea spaghetti (SS) and Irish wakame (IW)) and red (dulse (PP) and nori) Irish seaweed species represent potential nutritional sources of protein, amino acids, dietary fibre, minerals and health–promoting bioactive antioxidant compounds in the human diet. These seaweed species were found to contain low-fat levels, reducing their caloric content when ingested alone or as a food product component (Chapter 3).
- Brown seaweed species are significant sources of minerals (Na, Mg and Ca), total and insoluble dietary fibres. In contrast, red seaweed species possess superior protein content, phosphorus, and trace mineral levels (Chapter 3).
- Sea spaghetti and nori displayed the highest TPC and *in vitro* antioxidant activity, indicating the suitability of these seaweed species as superior sources of bioactive compounds in the diet or as ingredients for use in the manufacture of functional food products (Chapter 3).
- Addition of seaweeds did not significantly affect the proximate composition of fresh pork sausages. However, addition of seaweeds (nori, SS and IW) at a 5% inclusion level and PP at 2.5% and 5.0% negatively influenced pork

sausage's colour, instrumental textural properties and sensory attributes (Chapter 4).

- Regardless of the impact that seaweed addition had on sausage colour, panellist liking of product appearance was comparable to that of control samples. From the sensory results of seaweed sausages, consumers preferred sausages with lower seaweed content, and liking of seaweed sausages was in the order nori > SS > IW > PP (Chapter 4).
- The maximum acceptable inclusion level in pork sausages for PP was 1.0% and 2.5% for the other seaweed species (SS, IW and nori) (Chapter 4).
- Panellists accepted the reduced-fat and -salt brown seaweed sausages equally ($P<0.05$) at the higher fat and salt control. This result suggests that the brown seaweeds masked the variation of fat and salt in pork sausages (Chapter 5).
- Based on sensory results, the selected sausage formulations using SS, IW, PP and nori were 10% fat, 0.5% salt (SS10f/0.5s), 15% fat, 1% salt (IW15f/1.0s), 10% fat, 0.5% salt (PP10f/0.5s) and 10% fat, 1.0% salt (nori10f/1.0s), respectively (Chapters 5 and 6).
- In reduced- fat and -salt brown seaweed sausages (SS10f/0.5s and IW15f/1.0s), MAP70/30 and VP were the most effective packaging approaches in controlling lipid oxidation and microbial growth, respectively (Chapter 5).
- The packaging, MAP70/30, was the most effective packaging condition in extending the shelf-life of reduced -fat and -salt and red seaweed sausages (PP10f/0.5s and nori10f/1.0s) (Chapter 6).

- Consumers disliked the reduced fat and salt seaweed sausages, and nori10f/1.0s was the least liked and acceptable formulation (Chapter 7).
- The bacon stimuli (0.08%) did not enhance the saltiness perception of reduced fat and salt sausage containing nori seaweed (nori10f/1.0s) (Chapter 8).
- The bacon stimuli (0.08%) had a positive influence on the saltiness flavour of reduced-fat and -salt sausage (C2B) (Chapter 8).

9.3. Future work

This thesis provides a foundation for future studies on fat and salt reduction in processed meat products utilising seaweeds and OISE strategy. Some studies that could be carried out in future are as follows;

- Further characterise brown and red seaweed species used in this study- antimicrobial properties, identify and quantify bioactives.
- Utilise the direct application of brown and red seaweeds used in this study's extracts as antioxidants and antimicrobials in processed meat products.
- Investigate the use of brown and red seaweed used in this study as fat and salt replacers in other processed meat products such as white pudding, black pudding, salami and frankfurters.
- Explore and investigate the potential of other MAP gas combinations to improve seaweed sausages' shelf-life (Lipid oxidation and Microbiological properties).
- Assess the application of OISE strategy using other meat-related stimuli in improving the overall flavour and acceptability of seaweed sausages.
- Utilise other fat and salt- related stimuli (OISE strategy) in reduced fat and salt processed meat products such as pork sausages.

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APPENDIX

Table S2.1. Consumers' responses to the question, "If you have ever consumed seaweed or a seaweed product, Why? Select all that apply."

Responses	Frequency (n)	Percent
Source of iodine; Source of omega 3 fatty acids; Health benefits; Source of dietary fibre; Taste and flavour	1	0.2
Other	204	31.5
Taste and flavour; Other	210	32.5
Source of iodine; Source of omega 3 fatty acids; Source of protein; Taste and flavour; Sustainability	1	0.2
Health benefits; Other	3	0.5
Source of iodine; Taste and flavour	3	0.5
Source of iodine; Taste and flavour; Sustainability	1	0.2
Health benefits	14	2.2
Source of iodine; Taste and flavour; Sustainability; Source of omega 3 fatty acids	1	0.2
Source of dietary fibre; Health benefits	2	0.3
Health benefits; Source of dietary fibre	1	0.2
Health benefits; Source of dietary fibre; Source of protein	1	0.2
Health benefits; Source of dietary fibre; Source of protein; Source of omega 3 fatty acids	3	0.5
Health benefits; Source of iodine	2	0.3
Health benefits; Source of iodine; Plant based (vegan/vegetarian)	1	0.2
Health benefits; Source of iodine; Plant based (vegan/vegetarian); Source of protein	1	0.2
Health benefits; Source of iodine; Taste and flavour; Plant based (vegan/vegetarian)	4	0.6
Health benefits; Source of iodine; Plant based (vegan/vegetarian); Taste and flavour; Source of dietary fibre; Sustainability	1	0.2
Health benefits; Source of iodine; Source of dietary fibre; Plant based (vegan/vegetarian); Sustainability	1	0.2

Health benefits; Source of iodine; Source of dietary fibre; Source of protein; Taste and flavour; Plant based (vegan/vegetarian); Sustainability	1	0.2
Health benefits; Source of iodine; Source of omega 3 fatty acids	2	0.3
Health benefits; Source of iodine; Source of omega 3 fatty acids; Plant based (vegan/vegetarian)	77	11.9
Health benefits; Source of iodine; Source of omega 3 fatty acids; Source of dietary fibre; Source of protein	1	0.2
Health benefits; Source of iodine; Source of omega 3 fatty acids; Source of dietary fibre; Source of protein; Sustainability	1	0.2
Health benefits; Source of iodine; Source of omega 3 fatty acids; Source of dietary fibre; Source of protein; Taste and flavour; Plant based (vegan/vegetarian); Sustainability	2	0.3
Health benefits; Source of iodine; Source of omega 3 fatty acids; Source of dietary fibre; Taste and flavour; Source of protein; Plant based (vegan/vegetarian); Sustainability	1	0.2
Health benefits; Source of iodine; Source of omega 3 fatty acids; Source of protein; Taste and flavour; Sustainability	1	0.2
Health benefits; Source of iodine; Source of protein; Plant based (vegan/vegetarian); Source of omega 3 fatty acids	1	0.2
Health benefits; Source of iodine; Source of protein; Taste and flavour; Plant based (vegan/vegetarian)	4	0.6
Health benefits; Source of iodine; Taste and flavour; Plant based (vegan/vegetarian); Sustainability	2	0.3
Taste and flavour; Sustainability; Source of iodine; Health benefits	2	0.3
Health benefits; Source of omega 3 fatty acids; Source of dietary fibre; Taste and flavour; Plant based (vegan/vegetarian)	1	0.2
Health benefits; Source of omega 3 fatty acids; Taste and flavour; Source of dietary fibre	1	0.2
Health benefits; Source of protein; Other	1	0.2
Health benefits; Source of protein; Taste and flavour; Sustainability	1	0.2
Health benefits; Sustainability; Source of omega-3 fatty acids	1	0.2
Taste and flavour; Health benefits	17	2.6
Health benefits; Taste and flavour; Plant based (vegan/vegetarian)	2	0.3

Health benefits; Taste and flavour; Plant based (vegan/vegetarian); Sustainability; Source of omega 3 fatty acids	1	0.2
Plant based (vegan/vegetarian); Health benefits; Source of dietary fibre; Taste and flavour; Sustainability	1	0.2
Plant based (vegan/vegetarian); Source of omega-3 fatty acids; Source of dietary fibre	1	0.2
Plant based (vegan/vegetarian); Source of protein; Taste and flavour	1	0.2
Plant based (vegan/vegetarian); Sustainability	1	0.2
Plant based (vegan/vegetarian); Sustainability; Health benefits	1	0.2
Taste and flavour; Plant based (vegan/vegetarian)	4	0.6
Source of dietary fibre; Plant based (vegan/vegetarian); Source of iodine	1	0.2
Source of iodine	2	0.3
Source of omega-3 fatty acids	3	0.5
Source of omega 3 fatty acids; Source of dietary fibre; Taste and flavour	1	0.2
Source of omega 3 fatty acids; Source of iodine; Health benefits; Source of dietary fibre; Taste and flavour; Sustainability	1	0.2
Source of omega 3 fatty acids; Sustainability; Source of dietary fibre; Source of protein; Health benefits	1	0.2
Sustainability	2	0.3
Sustainability: Plant based (vegan/vegetarian); Taste and flavour	2	0.3
Sustainability; Taste and flavour; Health benefits	3	0.5
Taste and flavour	41	6.3
Taste and flavour; Health benefits; Other	2	0.3
Taste and flavour; Source of iodine; Source of dietary fibre; Plant based (vegan/vegetarian)	1	0.2
Taste and flavour; Sustainability	2	0.3
Taste and flavour; Sustainability; Other	1	0.2
Total	647	100

Table S2.2. Consumers' responses to the question "If you have ever consumed seaweed or seaweed products, which? Select all that apply."

Responses	Frequency (n)	Percent (%)
Other	4	0.6
I have not eaten any of these	49	7.6
Seaweed bread; Seaweed flakes; Seaweed crackers; Seaweed sushi rolls; Other	1	0.2
Raw seaweed	62	9.6
Raw seaweed; Seaweed bread	22	3.4
Raw seaweed; Seaweed crackers	1	0.2
Raw seaweed; Seaweed crackers; Seaweed supplement; Seaweed flakes; Seaweed bread	1	0.2
Seaweed flakes; Raw seaweed	38	5.9
Raw seaweed; Seaweed sushi rolls	48	7.4
Raw seaweed; Seaweed sushi rolls; Seaweed crackers	2	0.3
Raw seaweed; Seaweed sushi rolls; Seaweed crackers; Seaweed bread; Seaweed supplement	4	0.6
Seaweed flakes; Seaweed crackers; Seaweed sushi rolls; Raw seaweed	42	6.5
Raw seaweed; Seaweed sushi rolls; Seaweed crackers; Seaweed flakes; Other	4	0.6
Raw seaweed; Seaweed sushi rolls; Seaweed crackers; Seaweed flakes; Seaweed bread	1	0.2
Seaweed sushi rolls; Seaweed crackers; Seaweed flakes; Seaweed pasta; Raw seaweed	2	0.3
Raw seaweed; Seaweed sushi rolls; Seaweed crackers; Seaweed flakes; Seaweed pasta; Seaweed bread	1	0.2
Raw seaweed; Seaweed sushi rolls; Seaweed crackers; Seaweed flakes; Seaweed supplement	1	0.2
Raw seaweed; Seaweed sushi rolls; Seaweed crackers; Seaweed pasta; Seaweed bread	1	0.2

Raw seaweed; Seaweed sushi rolls; Seaweed crackers; Seaweed supplement; Seaweed pasta; Seaweed bread; Seaweed meat products; Seaweed flakes	1	0.2
Raw seaweed; Seaweed sushi rolls; Seaweed flakes	36	5.6
Raw seaweed; Seaweed sushi rolls; Seaweed flakes; Seaweed crackers	1	0.2
Raw seaweed; Seaweed sushi rolls; Seaweed supplement; Seaweed crackers	1	0.2
Seaweed crackers	5	0.8
Seaweed crackers; Seaweed flakes; Raw seaweed	1	0.2
Seaweed sushi rolls; Seaweed crackers; Seaweed flakes	51	7.9
Seaweed flakes	7	1.1
Seaweed flakes; Dried seaweed	1	0.2
Seaweed flakes; Seaweed crackers; Seaweed supplement; Seaweed bread	1	0.2
Seaweed flakes; Seaweed pasta; Raw seaweed	1	0.2
Seaweed flakes; Seaweed supplement	37	5.7
Seaweed pasta; Seaweed crackers; Seaweed supplement	1	0.2
Seaweed supplement; Raw seaweed	1	0.2
Seaweed supplement; Seaweed pasta	1	0.2
Seaweed sushi rolls	146	22.6
Seaweed sushi rolls; Other	1	0.2
Seaweed sushi rolls; Seaweed crackers; Seaweed bread	1	0.2
Seaweed sushi rolls; Seaweed crackers; Seaweed flakes; Seaweed pasta; Seaweed bread; Other	1	0.2
Seaweed sushi rolls; Seaweed crackers; Seaweed flakes; Seaweed supplement	5	0.8
Seaweed sushi rolls; Seaweed crackers; Seaweed meat products	18	2.8

Seaweed sushi rolls; Seaweed crackers; Seaweed supplement	1	0.2
Seaweed sushi rolls; Seaweed flakes	6	0.9
Seaweed sushi rolls; Seaweed meat products	1	0.2
Seaweed sushi rolls; Seaweed pasta	1	0.2
Seaweed sushi rolls; Seaweed pasta; Seaweed bread	35	5.4
Seaweed sushi rolls; Seaweed pasta; Seaweed bread; Other	1	0.2
Total	647	100

Table S3.3. Consumers' responses to the question, "If you have not consumed seaweed, why? Select all that apply."

Responses	Frequency (n)	Percent (%)
Other	15	2.3
Allergy reasons	15	2.3
Availability	167	25.8
Availability; Never had the opportunity to	49	7.6
I do not try new foods	22	3.4
I do not try new foods; It is unpleasant (Taste, smell, texture and appearance); Never had the opportunity to	7	1.1
It is unpleasant (Taste, smell, texture and appearance)	69	10.7
It is unpleasant (Taste, smell, texture and appearance); I do not try new foods	7	1.1
It is unpleasant (Taste, smell, texture and appearance); Never had the opportunity to	14	2.2
Availability; Never had the opportunity; Other	7	1.1
N/A	15	2.3
Never had the opportunity to	252	38.9

Availability; Never had the opportunity to; it is unpleasant (Taste, smell, texture and appearance); I do not try new foods	8	1.2
Total	647	100