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Sensorial, cultural and volatile properties of dairy powders, yoghurt and butter from pasture and non-pasture cow diets.

Thesis presented by

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for the degree of

Doctor of Philosophy

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December 2022

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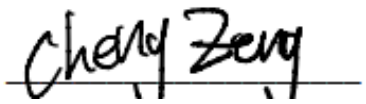
Dedication

This declaration 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.

Signature:



Cheng Zeng

14 December 2022

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Abstract

Significant advances have occurred in the ability to extract and identify volatile aromatic dairy compounds that may influence sensory perception. In this Thesis volatile extraction techniques were optimised and evaluated in order to obtain a more representative volatile profile of selected dairy products, such as; whole milk powder, skim milk powder, yoghurt and salted butter. This information was also utilised with gas chromatography olfactometry (GC-O) and sensory analysis to determine which specific compounds are most likely influencing sensory perception. In addition the impact of cow diet was assessed in terms of the volatile and sensory profile on skim milk powder, whole milk powder and salted butter using milk from cows outdoors on pasture-fed diets (such as ryegrass, ryegrass and white clover) and cows indoors on trial mixed rations. Cross cultural sensory analysis was also undertaken on skim milk powder from these diets in order to determine if consumers and trained panellists perceived skim milk powder differently based on diet but also on product familiarity. Finally, cross cultural sensory analysis was undertaken on yoghurt produced with different cultures in order to determine if any cultural preference's existed between German and Irish consumers and assessors, and if so what are the likely contributing factors?

Chapter 1 provides an updated review of traditional and novel sensory methods used to evaluate milk, milk powders, yoghurt, and butter, as well as gas chromatography mass spectrometry and gas chromatography olfactometry extraction techniques to provide more complete profile of volatiles that impact sensory perception. **Chapter 2** investigates the impact of pasture and non-pasture cow diets on the volatile cross cultural sensory perception of skim milk powder. The volatile profile and sensory properties of the skim milk powder were influenced by cow diet

and Irish, Chinese and USA consumers as well as trained sensory panellists perceived products differently primarily based on familiarity. **Chapter 3** investigated if yoghurt produced from three different starter cultures were perceived differently by Irish consumers, German consumers and trained assessors. Overall, there was no significant difference between the yoghurt products between German and Irish consumers, although Irish consumers rated the one sample significantly higher for 'liking of texture' ($p<0.01$) and two others significantly higher for 'liking of flavour' ($p<0.01$). However, trained assessors found significantly differences in over 50% of the 22 attributes evaluated. German trained assessors found it more difficult to discern differences between some of the yoghurts than trained Irish assessors. Seventeen of the 24 volatiles compounds identified differed due to starter culture, which most directly or indirectly associated with lipid oxidation.

The ability of headspace solid phase microextraction, thermal desorption, and high capacity sorptive extraction as a direct immersion and headspace extraction technique were compared with and without salting out and by a polar and non-polar gas chromatograph column for volatile profiling of whole milk powder in **Chapter 4**. Gas chromatograph column polarity significantly impacted volatile recovery, where salting out had a minimal impact. The range, recovery, reproducibility of volatiles were significantly impacted by each extraction technique. Direct Immersion high capacity sorptive extraction extracted the largest volume number and of volatiles, however only a combination of all extraction techniques could provide the most representative volatile profile.

The impact of three different diets on the sensory properties and volatile profile of whole milk powder was investigated in **Chapter 5**. Both the sensory perception and volatile profiles of whole milk powder differed significantly depending on the diet,

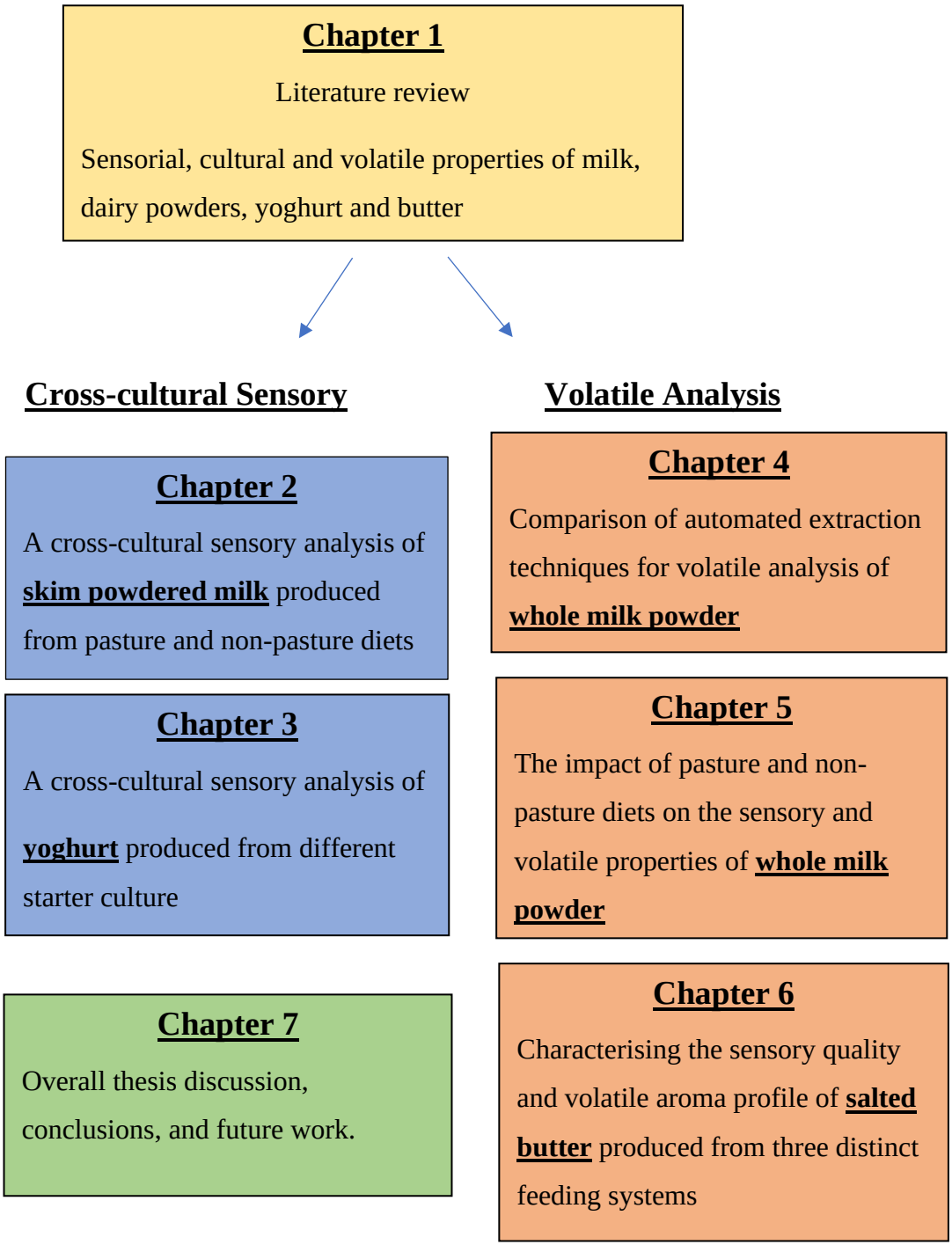
with whole milk powder derived from rye-grass or rye-grass and white clover more similar than whole milk powder derived from total mixed ration. Most of the differences in volatiles due to diet were either directly or indirectly linked to fatty acid content. **Chapter 6** outlines the development and optimisation of direct immersion high capacity sorptive extraction for the extraction, separation and identification of volatile compounds from salted butter from three different diets; rye-grass or rye-grass and white clover or from total mixed ration. This method was used for volatile profiling and for gas chromatography oflactometry using a trained panel. Over 60 % of the volatiles detected were influenced by diet, and directly and indirectly related to fatty acids. Twenty-four distinct odour activities differed significantly based on diet, with salted butter derived from both pasture diets have more intense and complex odours.

This thesis has clearly demonstrated that cow diet influences the volatile and sensory characteristics of selected dairy products, which subsequently effects sensory perception on a cultural basis influenced by product familiarity. The benefits of optimising volatile extraction techniques on a product specific basis were clearly demonstrated along with using multiple techniques in order to achieve the most representative volatile profile as possible. Combining volatile analysis with olfactometry and / or sensory techniques enables a more comprehensive understanding of factors influencing sensory perception and choice that can be utilised for product quality, improvement and marketing.

Thesis Objectives

Thesis Objectives

The relationships between the objectives of individual chapters and the overall objective of this thesis are presented in the figure below.



Publications

List of Publications

Peer Reviewed Journals

1. **Cheng, Z**., O'Sullivan, M. G., Kerry, J. P., Drake, M. A., Miao, S., Kaibo, D., and Kilcawley, K.N. 2020. A cross-cultural sensory analysis of skim powdered milk produced from pasture and non-pasture diets. Food Research International. 138: 109749.
2. **Cheng, Z**., Mannion, D.T., O'Sullivan, M.G., Miao, S., Kerry, J.P., and Kilcawley, K. N. 2021. Comparison of automated extraction techniques for volatile analysis of whole milk powder. Foods. 10: 2061.
3. **Cheng, Z**., O'Sullivan, M. G., Miao, S., Kerry, J. P., and Kilcawley, K. N. 2022. Sensorial, cultural and volatile properties of milk, dairy powders, yoghurt and butter: A review. International Journal of Dairy Technology.
4. **Cheng, Z.**, Mannion, D.T., O'Sullivan, M.G., Kerry, J.P., Miao, S., and Kilcawley, K.N. 2022. The impact of pasture and non-pasturediets on the sensory and volatile properties of whole milk powder. Journal of Dairy Research. 2022: 1-14.

Manuscripts in preparation

- **Cheng , Z.**, Mannion, D.T., Hennessy, D., O'Callaghan, T.F., O'Sullivan, M.G., Kerry, J.P., Miao, S., and Kilcawley, K.N. 2023. Optimization of a direct immersion high capacity sorptive extraction gas chromatography mass

spectrometry method to determine the impact of cow feeding systems on the volatile and aromatic properties of salted butter.

- ***Conference Presentations***

Oral

- Cheng, Z., O'Sullivan, M.G., Kerry, J.P., Drake, M.A., Miao, S., Kaibo, D., and Kilcawley, K. N. "A cross-cultural sensory analysis of skim milk powder produced from pasture and non-pasture diets". ADSA(American Dairy Science Association) 2019 Annual Meeting, Cincinati, Ohio, USA, June 23-25th 2019.
- Cheng, Z., Mannion, D.T., O'Sullivan, M.G., Kerry, J.P., Miao, S., and Kilcawley, K.N. "Comparison of different volatile extraction techniques for whole milk powder". 48th Annual Food Science and Technology Conference hosted by University of Limerick (UL) and the IFSTI, December 16th 2019.

Poster

- Cheng, Z., O'Sullivan, M.G., Kerry, J.P., Drake, M.A., Miao, S., Kaibo, D., and Kilcawley, K. N. "Consumer perception of Irish skim milk powder: A cross-cultural study between Ireland, America and China". Grass-fed Dairy Conference, Osprey hotel, Naas, Co. Kildare, Ireland, October 25th 2018.
- Cheng, Z., O'Sullivan, M.G., Kerry, J.P., Drake, M.A., Miao, S., Kaibo, D., and Kilcawley, K. N. "Consumer perception of Irish skim milk powder: A cross-cultural study between Ireland, America and China". 8th European Conference on Sensory and Consumer Research, Verona, Italy, September 2-5 th 2018.

- Cheng, Z., O'Sullivan, M.G., Kerry, J.P., Drake, M.A., Miao, S., Kaibo, D., and Kilcawley, K. N. "Consumer perception of Irish skim milk powder: A cross-cultural study between Ireland, America and China ". 47th Annual Food Science and Technology Conference hosted by University College Cork (UCC) and the Institute of Food Science and Technology of Ireland (IFSTI), December 6-7 th 2018.
- Cheng, Z., Mannion, D.T., O'Sullivan, M.G., Kerry, J.P., Miao, S., and Kilcawley, K.N. "Comparison of four volatile gas chromatography mass spectrometry extraction techniques with and without salting out using both polar and nonpolar columns on whole milk powder". Irish Mass Spectrometry Society Conference. Virtual online, May 11- 12th 2021.

Awards

- Cheng, Z., Mannion, D.T., O'Sullivan, M.G., Kerry, J.P., Miao, S., and Kilcawley, K.N. "Comparison of four volatile gas chromatography mass spectrometry extraction techniques with and without salting out using both polar and nonpolar columns on whole milk powder". Irish Mass Spectrometry Society Conference. Virtual online, May 11- 12th 2021. 3rd place of the Best Poster award at the Irish Mass Spectrometry Society conference, May 12th.

List of abbreviations

%	Percentage
°C	Degrees celsius
µL	Microlitre
ANOVA	Analysis of variance
CAS	Chemical abstracts service
CCD	Central composite design
CLA	Conjugated linoleic acid
CLV	Perennial ryegrass/white clover
cm	Centimetre
DA	Descriptive analysis
DH2O	Distilled Water
DI	Direct Immersive
FFA	Free fatty acid
g	Gram
GCMS	Gas chromatography mass spectrometry
GC-O	Gas-chromatography olfactometry
GRS	Perennial ryegrass
h	hour
HS-SPME	Headspace solid phase microextraction
IS	Internal standard
kg	Kilogram
L	Litre
LRI	Linear Retention Index
min	Minute
mL	Millilitre
MS	Mass spectrometry
No.	Number
ODP	Optimized descriptive profiling
P&T	Purge and trap
PCA	Principal component analysis
pH	Potential hydrogen
PLS-DA	Partial least squares discriminant analysis
RDA	Ranking descriptive analysis
RSD	Relative standard deviation
RSM	Response surface methodology
SAFE	Solvent assisted flavour evaporation
SDE	Simultaneous distillation extraction
SMP	Skim milk powder
SPSS	Statistical package for the social sciences
TCATA	Temporal check-all-that-applies

TD	Thermal desorption
TMR	Total mixed ration
UFA	Unsaturated fatty acids
UK	United Kingdom
USA	United States
VOC	Volatile organic compound
W/v or wt/vol	Weight per Volume
WMP	Whole milk powder
CAR/DVB/PDMS	Carboxen/divinylbenzene/polydimethylsiloxane
KW-PLSR	Kruskal wallis partial least squares regression
VIP	Variable importance plot
UHT	Ultra-high temperature
HTST	High temperature short time
SCFFA	Short chain carboxylic acids
HCA	Hierarchical clustering analysis
HiSorb	High capacity sorptive
CCRD	Central composite rotatable design
PUFA	Polyunsaturated fatty acids
OAC	Odor active compounds
OI	Odor intensity
LAB	Lactic acid bacteria
PSLR	Principal Component Analysis (PCA) and multiple regressions

Thesis Introduction

The increased interest worldwide by consumers in eco-friendliness, animal welfare, and food origin is reflected in product choice and has seen a rise in traditional authentic natural products over recent years. This has benefitted exports of Irish dairy products as Ireland is in a relatively unique position as one of the few bovine pasture based milk production systems globally. Fresh pasture significantly alters the fatty acid profile of milk, increasing the concentration of health promoting omega-3 fatty acids such as conjugated linolenic acid (CLA) and β-carotene content, which enhances yellowness, most obvious in by the colour of butter produced on this type of feeding system. It has been established that feeding systems also influence fat and protein content, soluble calcium, vitamins and volatile content, all of which may also impact directly or indirectly influence the sensory perception of milk and subsequent dairy products.

Volatile organic compounds (VOC) directly and indirectly derived from bovine diet are potentially very important components that influence sensory perception. The most significant components of flavour arise from these aromatic compounds, more so than taste as they can be perceived both ortho- and retro-nasally. Most of the significant differences in relation to VOCs in dairy products are fatty acids, aldehydes, alcohols, ketones, lactones and esters. However, their impact on sensory perception is dependent on their relative concentration and odour activity. Pasture feeding can increase odour intensity and odour complexity compared to dairy products derived from concentrate diets.

Thus, it is imperative that a better understanding of how differences between pasture based and feed or concentrate based feeding systems influence VOC content and abundance in milk and dairy products, and how these are perceived globally but especially in important markets for Irish dairy products, where consumers are familiar with pasture based dairy product systems, such as the USA, or not familiar with dairy products at all, such as China.

Different food environments and dietary experiences across cultures are known to influence both sensory perception and consumer behaviour. Therefore, the understanding of dairy product familiarity by consumers and cultural differences associated with food perception is essential to understand differences in consumer behaviour. Cross-cultural studies assist in understanding how consumers from different cultures perceive foods and therefore can assist in achieving greater market penetration, as it involves both consumer psychology and the dynamic interaction between the consumer, the context, and the dairy products. Thus identifying key odour active volatiles impacted by bovine diet in selected dairy products and correlating this data with cross cultural sensory analysis can potentially provide information that could lead to targeting of specific dairy products for selected global markets, by having a more precise understanding of cultural perceptions in relation to dairy products derived from pasture and non-pasture based feeding systems.

A key element in better understanding the VOC profile of milk and dairy products is having the necessary capability and expertise to achieve this. The flavour chemistry facility at Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork is the leading such facility in Ireland and is recognised internationally. Thus, some of the most advanced automatic and semi-automatic volatile extraction techniques will be

utilised in combination with gas chromatography mass spectrometry and gas chromatography olfactometry to extract and profile the aromatic properties of selected dairy products in this study. Sensory analysis was managed within the School of Food and Nutritional Sciences at University College Cork, which has extensive expertise and facilities and collaborations with similar facilities in the USA, China and Germany that will be utilised in order to achieve the objectives of the thesis.

The aims of this thesis are therefore to identify key odour active VOC in selected Irish dairy products (whole and skim milk powder, butter, yoghurt and milk) and to employ cross-cultural sensory techniques to determine differences in perception between important global markets and cultures for these products.

The objective of the research undertaken in this thesis were to;

(i) Review the current literature in relation to volatile and sensory analysis of milk, dairy powders, yoghurt and butter (Chapter 1).

(ii) Review, utilise, compare and optimise selected volatile extraction techniques for dairy products (Chapters 2, 3, 4, 5, 6).

(iii) Identify the major volatiles compounds in skim milk powder, whole milk powder, yoghurt and butter (Chapters 2, 3, 4, 5, 6).

(iv) Generate scientific data on the association between volatile compounds and sensory characteristics of skim milk powder, whole milk powder, and yoghurt produced from pasture and non-pastured based feeding systems (Chapters 2, 4, 5 & 6).

(v) Determine cross-cultural sensory differences of selected Irish pasture dairy products between USA, Ireland, China and Germany (Chapters 2 & 3).

(vi) Identify key aromatic volatiles responsible for the odour of butter generated from pasture and non-pasture based feeding systems (Chapters 6).

Chapter 1. Sensorial, cultural and volatile properties of milk, dairy powders, yoghurt and butter: A review

This chapter has been published in *International Journal of Dairy Technology*

Abstract

Countries with an established dairy tradition consume milk, milk powder, yoghurt and butter directly or as an ingredient; however, in countries without this tradition the lack of familiarity and unknown expectations can be challenging to overcome. Therefore, having a better understanding of the volatile properties that influence their sensory appeal can aid overcoming these challenges. This review focusses on traditional and novel sensory methods used to research milk, milk powders, yoghurt and butter as well as the extraction techniques used in gas chromatography mass spectrometry and gas chromatography olfactometry to identify volatiles in these products that influence sensory perception

Keywords: Milk, Dairy powders, Yoghurt, Butter, Sensory, Volatile organic compounds.

1.1 Introduction

Sensory analysis is an important part of dairy product development and manufacture, providing answers to specific flavour, visual and textural characteristics, and hedonic consumer responses amongst others. Affective tests incorporating preference and hedonic testing use subjective criteria of untrained consumers to provide important market information cost effectively (Stone et al. 2020). Combinations of affective and analytical techniques (threshold, discrimination and descriptive tests) are applied to take advantage of each technique's convenience for specific purposes providing important sensory information that can be used for example to improve product quality and/ or market share. New sensory methodologies have been developed with the aim of rapidly providing sensory data more cost effectively, but doing so relatively simply in comparison to traditional techniques (Ruiz-Capillas et al. 2021). Such sensory methods include check-all-that-apply (CATA), flash profiling (FP), rate-all-that-apply (RATA) using trained panels or semi-trained panels.

Cross-cultural sensory and consumer research is becoming increasingly important, as it involves both consumer psychology and the dynamic interaction between the consumer, the context, and the food (Lee et al. 2010). Culture is one of the significant factors underlying consumers' food choices, influencing attitudes and beliefs about food (Rozin 1988). Different food environments and dietary experiences across cultures influence both sensory perception and consumer preferences (Prescott and Bell 1995). Cross-cultural studies aid in the understanding of how consumers from different cultures perceive foods and assists in achieving market penetration, especially for new products or for unfamiliar products in new markets (Ares 2018).

As food aroma is such a significant factor in flavour, it is a widely researched topic, with over 10,000 volatile organic compounds (VOC) known to exist with less than 3 % thought to contribute to the aroma of any given food (Dunkel et al. 2014). VOCs must be present at a concentration above their odour threshold in order to be perceived, this is typically measured as their Odour Activity Value (OAV) which is the ratio of the concentration to the odour threshold. In most cases the presence of multiple VOCs are essential for the characteristic aroma of a product, rather than a single VOC. It is also thought that many other factors not just the OAV of VOCs impact sensory perception, such as gustatory and trigeminal components and genetic differences between individuals etc (Spence 2021). However understanding the VOC profile of a food gives an important insight into the relationship between VOC and multisensory flavour perception, but determining the true VOC profile of any product is difficult due to the many factors that can impact analysis.

A key aspect of VOC research that is often not addressed in dairy research is the actual relationship between VOC and sensory perception. This can be achieved to some extent using multivariate statistical analysis of VOC and sensory data (ideally descriptive sensory data) where some plausible associations can be implied, however it is much more beneficial to undertake gas chromatography-olfactometry (GC-O) where individual aroma active VOC can be identified (Sarhir et al. 2021; Cadwallader and Singh 2009) as well as their potential significance to the overall aroma. There are several factors involved in the processing of dairy products that impact VOC; such as oxidative stability, thermal treatments, high pressure, ultrasound and addition of processing aids, ingredients, cultures or enzymes (Cadwallader and Singh 2009; Vazquez-Landaverde et al. 2005; Riener et al. 2009; Serra et al. 2009; Liu et al. 2022). Table 1 summarizes the composition of and common thermal treatments applied to, milk dairy

powders, butter and yoghurt during processing. This review will focus on the key aroma active VOC in milk, dairy powders, yoghurt and butter and their relationship to product quality from a flavour perspective, incorporating cross cultural sensory analysis and new trends in sensory science applicable to these products. This review did not include cheese due to the added complexity of the product, and the fact that so many studies have been undertaken that it would require a separate independent review.

Table 1.1 Summary of the processing method and compositional in milk, milk powder, yogurt and butter

Dairy products	Processing Method	Parameters Employed	Compositional Information (%)	Reference
Milk	UHT	135 °C for 2 to 5 s	Fat (%) : 3	a
	High temperature short time(HTST)	72°C for 15 s	Fat (%) : 4.6;Total solids (%): 13.88; Whey protein (%): 4.2	b,c,d
	Low temperature long time(LTLT)	63 °C for 30 min		d
	High hydrostatic pressure processing (HPP)	40 °C, 600 MPa for 5 min		d
	Ultrasound (US)	400 W, 45°C for 2.5 to 20 min		e
SMP	Low heat spray drying	71 °C for 20min	Moisture (%): 4.6	f,g
	Medium heat spray drying	71-79 °C for 20 min		
	High heat spray drying	90 °C for 30 min		
WMP	Medium-heat Medium-heat	65 °C for 20 min	Moisture (%): 2.5;Whey protein (%):5.4; Fat (%): 29	i,j
Yogurt	High temperature short time(HTST)	72°C for 15 s	Total protein (%):4.1; Fat (%): 4.3	k,l
	Ultra-high pressure homogenized (UHPH)	300MPa, 90 °C for 90 s		
Butter	High temperature short time(HTST)	72°C for 15 s	Moisture (%): 14; Fat (%): 83	m

The data adapted from

a	Vazquez-Landaverde *et al.* 2005	g	Turner *et al.* 2002
b	Faulkner *et al.* 2018	i	Clarke *et al.* 2020b
c	O'Callaghan *et al.* 2019	j	Lloyd *et al.* 2009
d	Liu *et al.* 2020b	k	Tian *et al.* 2017
e	Riener *et al.* 2009	l	Serra *et al.* 2009
f	Karagül-Yüceer *et al.* 2001	m	O'Callaghan *et al.* 2016

1.2 Sensory Analysis

1.2.1 Sensory Techniques

Sensory science is used to assess, study, and explain the response of the peculiarities of food that are observed by panellists using their senses of sight, smell, taste, touch, and hearing (Stone et al. 2020). Sensory analysis is used to obtain a better understanding of the relationship between aroma and sensory perception. Different types of sensory analyses, from conventional methods (Consumer Acceptance Testing and Quantitative Descriptive Analysis) to novel rapid sensory techniques (Check All That Apply, Flash Profile, Temporal Dominance of Sensations, etc.) are used to understand more about key sensory attributes and/or preferences of dairy products (Drake 2007; Andrewes et al. 2021).

Consumer acceptance testing is easy to preform using hedonic scales without sensory training. The hedonic scale assumes that participants' preferences exist on a continuum and that their responses can be categorized into the degree of liking or disliking of sensory attributes, such as appearance, odour, taste, aroma, texture (O'Sullivan 2016). The most widely used scale for measuring food acceptability is the 9-point hedonic scale, which has ruler-like and equal-interval properties with 'dislike extremely' on the left and 'like extremely' on the right (Wichchukit and O'Mahony 2015). Previous sensory studies have employed between 18 to 310 consumers for hedonic testing of milk, butter, yogurt, and dairy powders (Potts et al. 2017; Cheng et al. 2020; Garvey et al. 2020; Hoppert et al. 2013; da Silva et al. 2021; Clarke et al. 2020a).

Descriptive tests consist of a full sensory description of the products and require fewer panellists, but the panellists must be highly trained to distinguish

between attributes previously selected through focus groups (using selected sensory attributes from product references or standards) that best describe the product, and to evaluate their perception with quantitative values (O'Sullivan 2016). Quantitative descriptive sensory analysis (QDA) is one of main descriptive analysis techniques in sensory evaluation. Clark et al. (2020b) used 12 trained (60 h) descriptive sensory panellists to assess milk samples from different diets and the results of full descriptive sensory analysis provided a reliable insight into the differences of milks based on cows feeding system. However, operating traditional descriptive trained panels is expensive and time-consuming, and therefore other methods have been developed in order to obtain sufficient sensory information, but more rapidly and cost effectively.

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). Cheng et al. (2020) used ODP method to identify the sensory attributes of skim milk powder (SMP) produced from different cows diets with trained assessors from Ireland and China. Irish and Chinese trained assessors had different preferences for many attributes, and both found it more difficult to discern differences between SMP derived from cows outdoors fed perennial ryegrass or perennial ryegrass with white clover, than SMP produced from cows indoors on a concentrate diet.

CATA is another sensory approach to rapidly assess products. Consumers are presented with the sample and a versatile multiple-choice questionnaire, then asked to indicate which words or phrases appropriately describe their sensory experience (Ares et al. 2015). The terms might include sensory attributes, hedonic responses, emotional responses, purchase intentions, potential applications, product positioning, or other terms that the consumer might associate with the sample. Harwood and Drake (2020)

used a list of 22 features in a CATA format to identify what features typically influenced panellists purchase of milk. The results demonstrated that consumers generally expressed preferences that aligned with their explicit beliefs, and flavour considerations appeared to be a secondary differentiator of preference.

FP is another rapid low cost technique where untrained panellists select their own terms to describe and evaluate a set of products simultaneously, and then rank the products for each attribute that they individually create. Panellists are forced to generate discriminative attributes of the whole sample set but not on a hedonic term (Delarue 2015). Yao et al. (2018) used FP with 17 sensory attributes developed by 10 panellists for yoghurts produced by pasteurisation or by thermisation. FP was able to discriminate yoghurts based on the heat-treatment applied.

Temporal dominance of sensations (TDS) is dynamic descriptive sensory technique that involves repeatedly assessing, until the sensations end, and determining which sensation is dominant in scoring its intensity (Pineau et al. 2009). Compared to time–intensity, this method considers the multidimensionality of the perceptual space over time. Hutchings et al. (2017) used TDS to analysis milk protein hydrolysates using 20 consumers over 6 training sessions. Similar TDS results were obtained by the panellists from three levels of training session (untrained, familiarized and trained) for each product, but training also increased panel consensus and the ability to discriminate between milk protein hydrolysates. As the training session increased, the number of attributes selected decreased and the time spent on a given attribute increased.

1.2.2 Cross Cultural Sensory Analysis

The familiarity of food products plays an important role in acceptability and preference because it decreases the uncertainty about the safety and suspense associated with a novel product by generating a better match between expectations and sensory characteristics (Borgogno et al. 2015; Methven et al. 2012). For several studies, familiarity has had a positive effect on the liking scores of the food items and demonstrates a products' palatability and safety (Torrico et al. 2019; Prescott 1998). Liem et al. (2016) noted that Chinese consumers who had repeated exposure to the taste of ultra-high temperature (UHT) milk preferred UHT milk over pasteurised milk, highlighting that familiarity is a powerful driver of consumer liking. Cross-cultural differences exist in that familiarity may even influence trained panellists' perception of an attribute, e.g to be more or less intense than it actually is when responding to unfamiliar products (Lee et al. 2010). Tu et al. (2010) also concluded that the French panels who were less familiar with soya yoghurts needed twice as many attributes to describe the product's aroma than a Vietnamese panel who were more familiar with these products. Garvey et al. (2020) investigated the liking and perception of salted butters, produced from milk derived from different diets (perennial ryegrass or perennial ryegrass and white clover, or concentrate) by consumers in Ireland, Germany and the USA. The results demonstrated that familiarity contributed to sensory differences in Irish butter identified by German, Irish and USA consumers and assessors. Irish consumers preferred the appearance and flavour of butters produced from milk derived from cows outdoors on perennial ryegrass or perennial ryegrass and white clover, than German and USA consumers. German consumers found the salt intensity highest in butter produced from cows milk derived from the perennial ryegrass or perennial ryegrass and white clover, which was thought to relate to the softer texture of these butters and their more rapid melting properties due to changes

in fatty acid content, as the salt contents were similar. Familiarity was also postulated to contribute to differences in ‘appearance liking’ and ‘colour liking’ of these butters by USA consumers, where the butter produced from milk derived from cows indoors fed on concentrate scored highest, as this is the most widespread type of this butter available in the USA.

Consumers may also rely on their memory associative structure created from past personal experiences to influence acceptance, which is heavily influenced by culture. The multidimensional experience (sensory perception, memory, culture, and emotions) by consumers may increase acceptance for products (Corredor et al. 2010). The appearance is the first attribute evaluated by consumers and the visual information of the samples strongly influences the hedonic scores (Zampini et al. 2007). Satisfaction of these extrinsic aspects can influence overall liking, and thus purchase intent and even willingness to pay a premium, particularly for dairy products (Bir et al. 2020; Scozzafava et al. 2020). Hay et al. (2021) investigated consumer sensory preferences for drinkable yoghurt and the impact of provenance using Chinese, European and New Zealand consumers. In terms of sensory drivers ‘sweetness’, ‘sourness’, ‘strawberry flavour’, ‘dairy flavour’, ‘creamy flavour’ and ‘creamy texture’ and ‘thickness’ were correlated with culture. Chinese consumers had a cultural expectation for higher levels of sweetness compared to New Zealand and European consumers, while New Zealand consumers expected higher level of sourness, but not too sour. Dairy flavour was an important sensory attribute for Chinese consumers, and expectations concerning ‘strawberry flavour’ and ‘thickness’ also differed between the cultural groups.

Novel or unfamiliar food products are usually rejected by consumers and consistently score lower liking scores for all sensory attributes regardless of the cultural group (Pingali 2007). Tan et al. (2015) contrasted two groups of potential consumers with and without cultural exposure to specific foods and found that rejection of unfamiliar foods was greater than familiar foods, which can be considered a big factor in product development of novel food items. Ethnic food in a cultural community is often regarded as novel food by another community (Bell et al. 2011). Cheng et al. (2020) assessed consumer perceptions of SMP produced from milk derived from cows outdoors fed perennial ryegrass, or perennial ryegrass and white clover, or cows indoors fed concentrate in Ireland, China and USA. Chinese consumers could not discern a difference between the three SMP produced from the different diets, but rated ‘aftertaste liking’ and ‘aftertaste intensity’ differently than Irish and USA consumers, which may relate to the fact that some attributes were difficult to categorise with ambiguous cultural meanings. Moreover, Chinese consumers and trained assessors scored many attributes quite differently than their Irish or USA counterparts, likely again reflecting a lack of familiarity with dairy products. USA consumers had preference for SMP produced from milk derived from cows on a concentrate diet, while Irish consumers generally preferred SMP produced from cows on a pasture diet (either perennial ryegrass or perennial ryegrass and white clover diets), which reflects the main sources of cow diet used in both geographical locations.

Situational interpretations and meanings can also differ across languages and cultures. This can be a problematic for panels (consumers) only measuring the momentarily blinded sensory perception for preference, liking and acceptance, by the fact that anchors have also been shown to influence cultural differences (Ares 2018;

Yeh et al. 1998). Sensory attributes do not necessarily have a direct relationship with a single ingredient and have not a direct translation across languages, and therefore can cause problems for consumers with dissimilar cultures and languages (Prescott et al. 1998). Cheng et al. (2020) suggested that differences in the ‘aftertaste liking’ attribute for Chinese consumers in relation to their perception of SMP may have more to do with the verbalisation of sensory perception and linguistic representation, rather than the Western definition of the term. A similar result was found by Zhi et al. (2016), where a high ‘aftertaste intensity of thickness and sweetness’ is often used as a positive term to describe better quality milk in China and thus the concept of ‘aftertaste’ may be cultural dependent, because the underlying conceptual elements and words used to describe its features may be dissimilar. Pingali (2007) identified that creamy attributes would not be considered a common descriptor to delineate the characteristics of dairy products in the Chinese and Korean language. Chinese and Korean groups would use goso/xiāng (fragrancy) instead to describe their perception of dairy products.

It is necessary to validate scales (especially the meaning and psychological properties of scale labels) and any questions within the cultures of interest properly before conducting any cross-cultural sensory evaluation. Instructions to participants and questions should be accurately translated from one language to the other by a bilingual person to ensure that they hold the same meaning across all the cultural groups under consideration, and to minimize differences in cultural interpretation and familiarity of any words (Helms 1992; Arnold and Smith 2013). Preference mapping can potentially allow the interpretation of preference data from another culture to be related to trained panel descriptions and measurements conducted in one's own language (Prescott 1998). Ares (2018) also suggested that the behavioural

measurements such as the Ranking or Best–Worst scaling becomes an alternative to hedonic scaling, which could decrease the mistranslation in scale-usages styles/response styles between Asian and Western consumers. Lee and Lopetcharat, (2017) highlighted that using a combination of behavioural measurements and sensometrics improved both the validity and reliability of cross-cultural sensory and consumer studies by both stabilizing the subjects' evaluative process and quantifying the effects of cultures. Kim et al. (2018) processed the verbal definition in conceptual elements of nutty with a sensory approach that correlates structured sensory space with cross-cultural sensory elements driving nuttiness perception. Their results revealed that each cultural group (Korean, Chinese and English-speaking-Western consumers) evaluated nuttiness in soymilk based on similar criteria, which avoided misunderstandings in sensory attributes caused by conceptual differences across culture. Köster and Mojet (2015) recommended the use of non-verbal methods, such as PrEmo (a tool used to measure the emotions evoked by materials) in cross-cultural research in order to overcome language differences in the use of emotional terms.

1.3 Volatile Profiling by Gas Chromatography Mass Spectrometry

1.3.1 Volatile Extraction Techniques

The VOC profile of dairy products can be influenced by animal diet, heat treatments, processing and storage conditions (Birchal et al. 2005; Baldwin et al. 1991; Kilcawley et al. 2018). As the composition of the dairy products varies extensively, this can have a significant impact on VOC extraction due to differences in VOC solubility in polar and non-polar phases within the product, and from interferences from other elements present, especially salts. These factors need to be taken into account to determine the most suitable method of extraction for their isolation and subsequent analysis (Jeleń et al. 2012). Many dairy products also contain active

microbes that are dynamically undergoing enzymatic and or chemical changes that both directly and indirectly impact on the VOC profile.

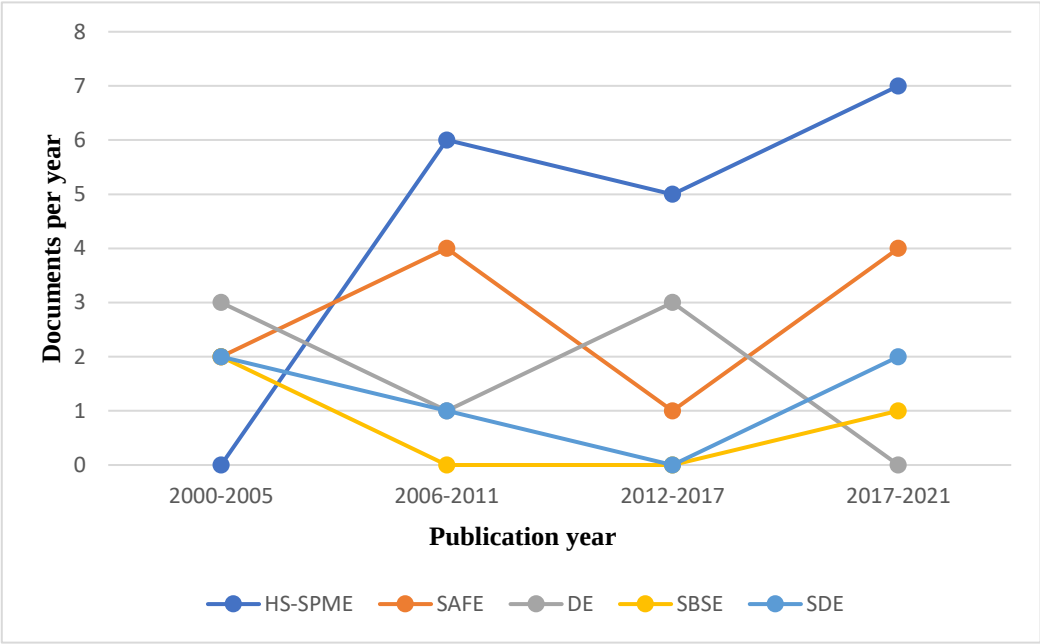
A wide array of extraction techniques have been employed to isolate and concentrate VOCs from different dairy products, including for example solid-phase microextraction (SPME) (Coppa et al. 2011; Cheng et al. 2020; Clark et al. 2019), solvent-assisted flavour evaporation (SAFE) (Evans et al. 2009), dynamic headspace extraction (DE) (Ciccioli et al. 2004), thermal desorption (TD) (Faulkner et al. 2018), stir bar sorptive extraction (SBSE) (Schiano et al. 2019) and simultaneous distillation extraction (SDE) (Kobayashi et al. 2008). However, reliable detection and complete quantification of VOCs in dairy products remains challenging (Schiano et al. 2019), as every technique has a degree of bias towards the extraction of certain chemical classes based aspects of the process itself, such as type of solvent or sorbent phase used (Ning et al. 2011). Therefore, it is best to utilise multiple extraction techniques if possible in an attempt to get the as true a volatile profile as possible for untargeted analysis. Most volatile extraction techniques are used in tandem with gas chromatography mass spectrometry (GC-MS), although other options exist, such as GC-FID (flame ion detection), SIFT-MS (selected ion flow tube mass spectrometry) and PTR−MS (proton-transfer reaction mass spectrometry) (Mariaca and Bosset. 1997; Olivares et al. 2011; Aprea et al. 2009).

Microextraction methods that have a minimal amount of extractant phase enable fast sample preparation, high sensitivity and are more easily automatable, and are thus becoming more widely favoured for VOC characterization. In addition as ‘green chemistry’ techniques that are seen as more environmentally friendly which require little or no solvents are becoming increasingly favoured. Figure 1A shows the

results of Web of Knowledge search for extraction methods used in milk, SMP, whole milk powder (WMP), yoghurt and butter between 2000-2021. In total ~ 44 publications were identified, dominated by headspace solid phase microextraction (HS-SPME), with solvent assisted flavour extraction (SAFE) also widely used (Figure. 1A). When looking into the types of dairy products in which multiple extraction techniques were used for analysis of VOC, the biggest group was for milk, followed by SMP, WMP, then butter and finally yoghurt (Figure. 1B).

A summary of the all the extraction methods including extraction conditions for milk (milk powders), butter and yoghurt are provided in Table 2. A total of 303 VOCs were identified by various extraction methods including aldehydes (54), alcohols (65), ketones (36), organic acids (30), sulphur compounds (7), terpenoid compounds (21), carbonyl compounds (15), lactones (19), esters (43) and furans (12).

(A)


8
7
6
5
4
3
2
1
0
Documents per year
2000-2005
2006-2011
2012-2017
2017-2021
Publication year
HS-SPME
SAFE
DE
SBSE
SDE

(B)

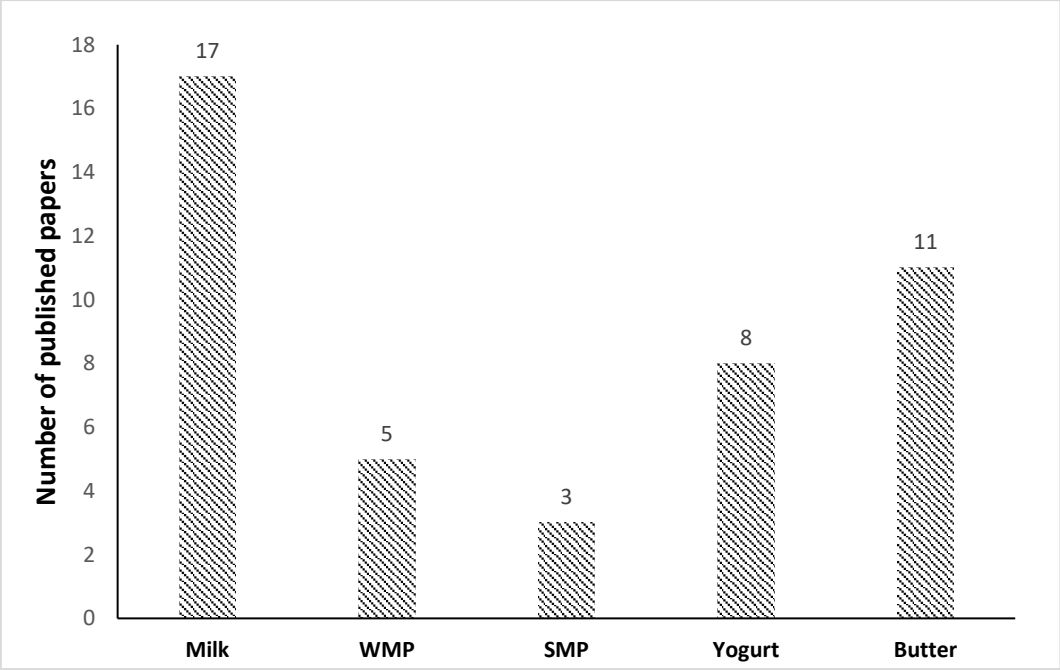


Figure 1.1 Applications of microextraction methods in selected dairy products. (a) Application of all extraction methods (HS-SPME, SAFE, DE, SBSE and SDE) used (b). Number of published studies based on Web of Knowledge search for years 2000–2021 incorporating all extraction techniques

Table 1.2 Volatile compounds found in milk (milk powders), butter, and yoghurt using GCMS by four common extraction methods

	Extraction methods				
Compound	**SPME**	**SAFE**	**DE**	**SBSE**	**REF**
Alcohols					
(E)-2-Hexen-1-ol		✓			a
(E)-2-Nonen-1-ol		✓			a
(E)-2-Octen-1-ol		✓			a
(E)-2-Octenal		✓			a
1,3-Butanediol		✓			b
1,4-Butanediol	✓				c
1-Butanol	✓	✓	✓		a,d,e
1-Dodecanol	✓				f
1-Heptanol	✓	✓	✓		a,g,h,I
1-Hexadecanol				✓	j
1-Hexanol	✓	✓	✓		a,e,i,j,l
1-Nonanol	✓	✓			a,I,k,l
1-Octadecanol		✓		✓	j,m
1-Octanol	✓	✓	✓	✓	a,h,I,j,l
1-Pentanol	✓	✓	✓		a,d,e,f,h,k,n,o
1-Penten-3-ol		✓			a
1-Phenylethanol				✓	j
1-Propanol		✓	✓		a,e,h
1-Tetradecanol				✓	j
2-(Methylthio)-ethanol		✓			a
2,3-Butanediol	✓	✓			a,k
2-Butanol	✓	✓			b,i
2-Ethyl-1-hexanol	✓				d
2-Ethylhexanol		✓			l
2-Furanmethanol	✓	✓	✓	✓	a,f,j,p
2-Heptanol		✓			b
2-Hexanol			✓		e
2-Methyl-1-butanol	✓				n
2-Methyl-1-propanol		✓	✓		a,e
2-Methyl-3-furanthiol	✓	✓			q,r
2-Methyl-3-pentanol	✓				i
2-Nonanol	✓				i
2-Octanol	✓				f
2-Pentanol		✓	✓		b,e
2-Pentanol, 4-methyl-			✓		e
2-Phenethanol		✓			r
2-Phenoxyethanol		✓			b
2-Propanol	✓				i
2-Propanol, 2-methyl-			✓		e
3-(Methylthio)-1-propanol		✓			a

(continued)

3-Hexanol	✓		✓		h,i
3-Methyl-1-butanol		✓			a,b
3-Methyl-2-butanol	✓				b,k
3-Methyl-2-hexanol		✓			b
3-Methyl-3-buten-1-ol	✓	✓			a,k
3-Octanol		✓			a
3-Pentanol	✓				k
3-Penten-2-ol	✓				k
4-Methyl-1-pentanol	✓				k
4-Methyl-2-pentanol		✓			b
4-Pentene-2-ol, 2-methyl			✓		e
Benzyl alcohol	✓	✓			a,d
Cyckobutanol	✓				i
Ethanol	✓	✓	✓		d,h,k,s,t
Ethyl furaneol	✓				u
Furaneol		✓			q
Furfuryl alcohol		✓			q
Heptanol	✓				i
Isomaltol				✓	j
Maltol		✓			w
Phenethyl alcohol		✓			a,l
Tetradecanol		✓			r
Thenylthiol		✓			r
Aldehydes					
(E)-2-Decenal		✓			a
Aldehydes					
2,4-Decadienal	✓		✓		g,p
2,4-Heptdienal, (E,E)	✓	✓			a,f,m
2,4-Hexadienal			✓		e
2,4-Nonadienal			✓		p
2,4-Nonadienal, (E,E)-	✓	✓			f,r
2-Decenal	✓		✓		f,p
2-Decenal, (Z)-				✓	j
Dodecanal				✓	j
Furaldehyde	✓				c
Heptanal	✓	✓	✓	✓	a,d,g,j,n,o,r,t,w,y,α
hept-cis-4-enal		✓			y
Hexadecanal		✓			a
Hexanal	✓	✓	✓	✓	c,g,I,j,m,n,p,r,t,w,x,y,β,γ
Hex-cis-3-enal		✓			y
Hex-trans-2-enal		✓			y
Methional	✓	✓			r,s,w,y
Nonanal	✓	✓	✓	✓	a,c,d,h,I,j,k,m,n,o,p,r,u,w,x,z,β
Octanal	✓	✓	✓	✓	g,j,m,p,r,z

(continued)

Pentadecanal		✓				a
Pentanal	✓	✓	✓	✓		d,g,j,n,r,t,α
Propenal, 3-phenyl-2-(Z)-	✓					f
Pyruvaldehyde		✓				y
Tetradecanal	✓	✓				a,β
Tridecanal		✓				δ
Undecanal	✓					g
Z-4-heptenal		✓				m,r,w,x
Carbonyl compounds						
2,4-Dimethylphenol		✓				m
2-Methoxy phenol		✓				m,r,δ
3-Ethyl toluene	✓					n
3-Methylphenol		✓				b
4-Ethylphenol		✓				m
Benzene	✓		✓			e,f
Benzene, ethyl-	✓		✓			g,h
Ethyl ether			✓			h
Methylbenzene	✓					c
o-Xylene	✓					n
Phenol	✓	✓			d,k,s,y	
P-xylene	✓					i
Styrene		✓				x
Toluene	✓		✓			d,e,h,f,k,n,t,z,α
Xylene, (m)-	✓		✓			e,f,n
Ketones						
1-Hexene-3-one		✓				m,r,x
1-Hydroxy-2-acetone	✓					c
1-Hydroxy-2-propanone	✓	✓	✓			b,d,p
2-Pentanone	✓		✓			e,g,I,k,n,t,z,α
2-Tridecanone	✓					I,k,s,β
2-Undecanone	✓	✓				a,d,f,I,k,q,s,β
3-Hexanone			✓			e
3-Hydroxy-2-butanone	✓	✓				a,c,β
3-Octanone		✓				o
3-Octen-2-one	✓					g
3-Pentanone			✓			e
3-Penten-2-one, 4-methyl-			✓			e
4-Decanone		✓				g
4-Ethylphenol		✓				y
4-Hydroxy-3-hex- anone	✓					k
4-Methyl-2-pentanone	✓	✓	✓			e,k,o
6-Methyl-5-hepten-2-one			✓			z
Acetoin	✓	✓				b,d,I,k,y

(continued)

Acetone	✓		✓		c,d,g,I,k,n,t,α
Acetophenone			✓	✓	h,j
2,3-butanedione	✓	✓			b,c,I,k,r,t,u,w,x,y,ε,η
Methyl heptanone	✓				i
Pentane-2,3-dione		✓			y
Lactones					
Geranylactone	✓				i
Sotolone		✓			r
γ-Butyrolactone		✓			l
γ-Crotonolactone				✓	j
γ-Decalactone	✓	✓			w,y,β
γ-Dodecalactone	✓	✓			w,y,β
γ-Hexadecalactone		✓			β
γ-Nonalactone	✓	✓			r,x,ε
γ-Octalactone		✓			r
γ-Tetradecalactone		✓			y
γ-Undecalactone		✓			a
ε-Caprolactone	✓				d
σ-Decalactone	✓	✓		✓	f,j,l,o,r,s,t,w,x,y,β,ε
σ-Dodecalactone	✓	✓		✓	a,b,d,f,j,m,o,q,β
σ-Hexalactone	✓	✓			o,t,w,y
σ-Octalactone	✓	✓			o,t,u,w,x,y
σ-Tetradecalactone		✓			y
σ-Undecalactone		✓			w
σ-Valerolactone		✓		✓	j,l
Sulphur compounds					
Dimethyl sulphide	✓	✓			k,r,t,x,η
Dimethyl sulfoxide		✓	✓		a,z
Dimethyl trisulphide	✓	✓	✓		q,r,w,x,y,z
Dimethyl disulphide	✓	✓	✓		m,r,s,z,α
Dimethyl sulfone	✓	✓			d,f,I,o,l
Dimethyl tetrasulphide		✓			t
Dipropyl disulphide	✓				q
Terpenoid compounds					
2-Carene	✓				v
2-Methylthiophene		✓			y
3-Carene	✓		✓		d,e,v
4-Terpineol			✓		e
Camphene			✓		e
Cresol-(p)	✓	✓		✓	f,w,y
Dehydro-p-cymene		✓			c
D-limonene	✓	✓			b,k,o
Limonene	✓	✓	✓		e,h,v,y,α,λ
p-Cymene	✓	✓	✓		f,o,v
r-Thujene		✓			y

(continued)

Sabinene			✓			e
Squalene				✓		j
Styrene			✓			z
Terpinolene	✓					v
α-Pinene	✓	✓	✓			d,e,t,v
α-Terpinene	✓		✓			e,v
α-Thujene			✓			e
β-caryophyllene		✓				y
β-pinene	✓	✓	✓			e,f,n,t,y
γ-Terpinene		✓				m
α-linalool		✓				l
Esters						
(E,E)-Farnesyl acetate		✓				r
2-Hydroxy-3-methyl-butanoic acid ethyl ester		✓				a
2-Hydroxy-propanoic acid ethyl ester		✓				a
2-Methylbutyl acetate		✓				r
3-Hydroxy-butanoic acid ethyl ester		✓				a
3-Phenylpropionate	✓					k
Acetic acid 2-phenylethyl ester		✓			a	
Butyl acetate	✓		✓			k
Butyl benzoate	✓					a,b
Ethyl 2-hydroxyhexanoate		✓				y
Ethyl 2-methylbutyrate		✓				y
Ethyl 3-methylbutyrate		✓				a
Ethyl 9-decenoate		✓				a
Ethyl acetate	✓	✓	✓			c,h,n,t,x,y
Ethyl butanoate		✓				b,r,x
Ethyl butyrate	✓					f
Ethyl decanoate		✓				f
Ethyl heptanoate			✓			h
Ethyl hexadecanoate	✓					β
Ethyl hexanoate		✓	✓			a,b,h
Ethyl lactate		✓				b
Ethyl nonanoate		✓	✓			a,h
Ethyl octanoate	✓	✓				a,b,n,r
Ethyl palmitate		✓				a
Ethyl tetradecanoate	✓					β
Ethyl tridecanoate		✓				a
Ethyl undecanoate			✓			h
Ethyl valerate			✓			h
Heptyl hexanoate	✓					f
Hexyl acetate			✓			h
Isopropyl palmitate				✓		j

(continued)

Methyl butanoate		✓			μ
Methyl dodecanoate	✓				a
Methyl heptanoate			✓		h
Methyl hexadecanoate	✓			✓	f,j
Methyl isobutyrate			✓		h
Methyl octanoate	✓				f
Methyl tetradecanoate	✓				β
Octyl formate	✓				c
Phenylethyl acetate		✓			r
Propyl benzoate	✓				c
S-methyl thio-3-methylbutyrate		✓			y
Furans					
2-Furanmethanol		✓			l
2-Pentylfuran	✓		✓		d,z
5-Butyldihydrofuran-2(3H)-one	✓				β
5-Ethyl-2(5H)-furanone	✓				β
5-Hydroxymethylfurfural				✓	j
5-Methyl furfural		✓			m
Benzofuran, 2,3-dihydro-2-methyl-	✓				f
Furan, 2-methyl-	✓				f
Furfural		✓	✓	✓	a,j,p
Homofuraneol		✓			m
Hydroxy-2(5)H-furanone				✓	j
Phenylacetaldehyde		✓			r
Acids					
2-Methylbutyric acid		✓			l
2-Methyl-propanoic acid		✓			a, b,l,w
3-Methylbutanoic acid		✓			b,w,x
3-Methylbutyric acid		✓			l,y
3-Phenylpropionic acid		✓			l
4-Methyl octanoic acid		✓			r
9-Decenoic acid		✓			W, l
Acetic Acid	✓	✓	✓	✓	a,b,c,d,h,I,j,k,m,n,q,r,s,w,x,y,ε,λ
Benzoic acid	✓	✓			c,f,k,l,j,β
Butanoic Acid	✓	✓		✓	a,c,d,f,j,I,n,q,r,s,w,x,ε,λ
Butyric acid	✓	✓			I,k,l,r,y,β
Decanoic acid	✓	✓		✓	a,b,c,f,I,j,k,i,l,m,o,r,s,w,β
Dodecanoic acid	✓	✓			a,f,l,o,w,β
Formic acid		✓			l,w
Heptanoic acid	✓	✓			a,b,c,k,l,w,i
Hexadecanoic acid	✓	✓			o,β
Hexanoic acid	✓	✓	✓	✓	a,b,c,f,j,k,l,m,n,p,s,t,x,β,ε

(continued)

Nonanoic acid	✓	✓		✓	a,b,c,f,j,k,l,o,t,β,ε
Octanoic acid	✓	✓		✓	a,c,f,I,j,l,k,o,s,w
Pentanoic acid	✓	✓	✓		a,b,I, h,,l, m,r,w, y
Phenylacetic acid		✓			l,y
Propanoic acid		✓			a,b,d,w,l
Tetradecanoic acid	✓	✓			a,f
Undecanoic acid	✓	✓			a,f,l,β

The data adapted from

a	Ning *et al.* 2011	r	Evans *et al.* 2010
b	Sarhir *et al.* 2021	s	Dadali and Elmaci 2019
c	Su *et al.* 2017	t	Garvey *et al.* 2020
d	Cheng *et al.* 2020	u	Abilleira *et al.* 2010
e	Ciccioli *et al.* 2004	v	Mahajan *et al.* 2004
f	Coppa *et al.* 2011	w	Lozano *et al.* 2007
g	Clarke *et al.* 2019	x	Bendall 2001
h	Rabaud *et al.* 2003	y	Jansson *et al.* 2014
i	Tian *et al.* 2017	z	Contarini and Povolo 2002
j	Faulkner *et al.* 2018	α	Sarrazin *et al.* 2011
k	Tian *et al.* 2019	β	Panseri *et al.* 2011
l	Miyaji *et al.* 2021	γ	Evans *et al.* 2009
m	Smith *et al.* 2016	ε	Martin *et al.* 2011
n	O'Callaghan *et al.* 2016	η	Salum and Erbay 2019
o	High *et al.* 2019	λ	Guneser and Yuceer 2011
p	Francesca *et al.* 2015		
q	Mallia *et al.* 2014		

1.3.2 Solid-Phase Microextraction

SPME is a widely used extraction technique, in part due to its relative simplicity (no extensive sample preparation) when compared to other techniques such as dynamic headspace extraction or solvent extraction. It is a manual or fully automotive technique (in conjunction with a robotic autosampler) and offers high reproducibility and is relatively inexpensive as SPME fibres can be used multiple times. SPME can be performed as a direct immersion procedure (DI-SPME) by exposing a phase-coated fibre into a liquid sample (Mallia et al. 2005), or as a headspace procedure (HS-SPME) (Pawliszyn 1997; Januszkiewicz et al. 2008). DI-SPME is not that widely practiced for dairy products, due to the fact that it can adversely impact on the longevity of the fibres due to repeated swelling and drying, and fouling of the fibre can also occur which also adversely impacts on the ability of low molecular weight VOCs interaction with fibre phases (Heaven and Nash 2012). A main advantage of SPME is that a wide range of sorbent phases are available; from single phases; polydimethylsiloxane (PDMS), and polyacrylate (PA); dual phases; carboxen (CAR)/PDMS, PDMS/divinylbenzene (DVB), carbowax polyethylene glycol (CW-PEG), CW/DVB, CW/TR (templated resin), or as triple phases; DVB/CAR/PDMS (Jeleń et al. 2012; Heaven and Nash 2012; Mondello et al. 2005; Yu et al. 2008). The type of fibre coating and thickness determine the properties in terms of polarity and retention, which affects the extraction efficiency, selectivity, reproducibility and discrimination of the extraction (Spietelun et al. 2010). A range of film thicknesses are also available; PDMS at 100, 30 and 7 μm, PA at 85 μm, PDMS/DVB at 65 and 60 μm, CAR/PDMS or CW/DVB at 75 and 65 μm and CW/TR at 50μm.

The most convenient way to discuss the coating capacity independent of its characteristics as a solid or liquid sorbent is to consider the fibre constant as defined by Rivellino et al. (2013), where:

$$Fc = KfsVf$$

Fc = fiber constant

Kfs= the, fiber coating/sample matrix distribution constant of the analyte

Vf = volume of the extraction phase

HS-SPME extraction is considered complete when the analyte concentration has reached a distribution equilibrium between the sample, headspace and the fibre (Mondello et al. 2005). The efficiency of adsorptive extraction is dependent on the analyte surface concentration in the extraction phase at equilibrium and the surface area of the extraction phase (Musteata and Pawliszyn 2005). During the extraction process the volume of VOC absorbed by the fibre phase is much faster than its release from the matrix, thus the requirement for sufficient time to obtain a representative VOC profile (Zabaras and Wyllie 2001). The length of extraction time and temperature are critical for SPME extraction efficiency. Generally, longer extraction times and high temperatures benefit the equilibrium resulting in increased responses of less volatile analytes (Fang and Qian 2005). However, care must be taken not to lose, create or enhance some VOC by the application of thermal treatments. The selectivity of HS-SPME is impacted by the selectivity of different phases towards specific solutes and various degrees of polarities. For example larger less volatile compounds are captured by the porous DVB phase, while lower molecular weight highly volatile compounds are captured by the porous CAR layer (Garcia-Esteban et al. 2004). DVB is composed of polymerized alkyl chains with phenyl groups creating a porous phase that is used

in combination with PDMS to aid in attaching the DVB and to increase selectivity (Heaven and Nash 2012). The PDMS phase tends to capture low to medium polarity compounds, with PA more suitable for highly polar compounds (Mondello et al. 2005). PA fibres are made of partially crosslinked acrylic acid monomers and swell slightly in water (Heaven and Nash 2012). CAR consists of different sized pores that capture compounds and are used in combination with PDMS as this helps attach the CAR to the fibre but also enhances selectivity (Heaven and Nash 2012). CW/DVB has ability to extract a wide range of low- to mid-molecular weight molecules (Carpino et al. 2004).

Merkle et al. (2015) mentioned that the binding of analytes to the matrix resulted in low concentrations of the analytes in the headspace in complex food matrices. Thus the matrix effect is worth considering when developing a HS-SPME or any HS method for the extraction of VOC in dairy products. However, high temperatures during extraction can reduce the adsorption ability of SPME fibre for the target analytes because the adsorption of fibre is an exothermic process (Ng et al. 1999). Generally, longer extraction times and high temperatures benefits the equilibrium and increases the responses of less volatile analytes, but often at the cost of sensitivity and possibly increase artifact formation (Mariaca and Bosset 1997). However, the quantification of sulphur VOCs can only be achieved under non-equilibrium conditions using short extraction time, particularly for complex matrixes due to their inherent instability (Nielsen and Jonsson 2002; Murray, 2001). In certain cases, low extraction efficiencies are reported, in particular for very volatile, polar, or thermally unstable analytes (Namieśnik et al. 2000). This is likely related to the relatively low capacity of the sorbent phases on the fibre in comparison to many other sorbent type extraction techniques.

Most HS-SPME studies involving milk, yoghurt, butter or dairy powders have used the three phase fibres. DVB/CAR/PDMS is particularly useful for the detection of highly volatile sulphur, alcohol, terpenes, esters and acid compounds (Abilleira et al. 2010). However, the overall recovery of more polar compounds, especially free fatty acids by the DVB/CAR/PDMS fibre is poor (Mondello et al. 2005). Tian et al. (2017) used a 50-µm DVB/CAR/PDMS fibre to extract VOCs in yoghurt. These authors found that an extraction/equilibration time of 40 min at 55 °C extracted 45 VOCs (aldehydes, alcohols, ketones, organic acids, and sulphur compounds), with ketones and aldehydes the most abundant chemical classes, followed by alcohols, acids, and sulphur compounds. Tian et al. (2019) subsequently extracted 54 VOC also in yoghurt samples using this same HS-SPME procedure (extraction time 40 min at 55 °C) and fibre. Ketones, aldehydes and alkanes were the most abundant chemical classes followed by alcohols, acids, carbonyl compounds and sulphur compounds. O'Callaghan et al. (2016) investigated VOCs in sweet cream butter derived from cows milk using a 75-µm DVB/CAR/PDMS fibre. The butter was equilibrated at 40 °C for 10 min, then the fibre was exposed to the headspace for a further 20 min at 40°C. In total 25 VOC were extracted consisting of aldehydes, ketones, alcohols, acids, esters, a terpene and toluene, p-xylene and phenol. Garvey et al. (2020) investigated VOCs in salted butter using an optimised HS-SPME method with a 50/30 µm DVB/CAR/PDMS fibre, with a pre-equilibration of 10 min at 40°C, followed by a 60 min extraction time at 40 °C. They identified 30 VOCs consisting of aldehydes, ketones, acids, hydrocarbons, lactones, sulphur compounds, esters, alkenes and a terpene and alcohol compound. This study highlighted that aldehydes, ketones, acids, terpenes and lactones were the main chemical classes contributing to the volatile profile of butter. Mallia et al. (2014) identified VOCs (aldehydes, ketones, acids,

lactones, hydrocarbons, sulphur compounds and an alcohol) in sour cream butters from different countries also using a 50/30μm DVB/CAR/PDMS fibre with an extraction temperature of 45°C for 45 min. Cheng et al. (2020) also used a 50/30 μm DVB/CAR/PDMS fibre to extract VOCs from SMP with an equilibration/extraction temperature of 40°C for 20 min. These authors extracted 26 VOCs (aldehydes, ketones, alcohols, terpenes, acids, a sulphur compound and a phenyl compound). Cheng et al. (2021) found the HS-SPME with DVB/CAR/PDMS appeared to be very effective at recovering terpenes and sulphur compounds in WMP, but much less effective at recovering lactones, furans, and acids.

Clarke et al. (2019) optimized the extraction of VOCs associated with lipid oxidation in WMP (2.4 g made up with 3.5 mL distilled water). The authors used the 50/30μm DVB/CAR/PDMS fibre and found that an extraction time of 45 min at 43°C using 2.4g of sample gave achieved the best extraction efficiency for VOC recovery. For the vast majority of the VOC selected (aldehydes and ketones) the limits of detection (LOD) varied between 0.002 and 0.006 mg/L, with limits of quantification (LOQ) of 0.05 and 0.066 mg/L. The authors noted a matrix effect, which was due to the degree of interactions of VOC with the sample, which was more apparent for longer chain aldehydes, likely due to their affinity with the fat phase in the WMP. The authors also concluded that the influence of the sample amount is less important for the recovery of polar than for non-polar VOCs. Matrix interference is a major issue with VOC analysis in foods, but especially for lipophilic compounds (Abilleira et al. 2010). One main reason is that as the solubility of VOCs increases in a hydrophobic solvent, while the vapour-liquid partition coefficient decreases (Druaux et al. 1998). Abbilleira et al. (2010) also utilised the 50/30μm DVB/CAR/PDMS fibre to quantify terpenes in ewe's milk fat, using a pre-equilibration time of 10 min at 40°C, followed

by extraction at 40°C for 30min. The authors noted that the matrix effect was a main reason for the overall systematic error to quantify terpenes (mono- and sesquiterpenes) in milk fat by HS-SPME.

Coppa et al. (2011) extracted VOC from the cream of cows milk derived from a hay-based diet or from continuous grazing of pasture. They also used the DVB/CAR/PDMS fibre. In this study the cream was frozen and thawed in a HS vial at 60°C for 20 min in a water bath, and then incubated with the fibre exposed at 60°C for a further 20 min. Seventy five VOCs were identified and the study demonstrated that the DVB/CAR/PDMS fibre recovered VOCs with both high and low polarities. However, the relatively high temperature (60°C) used in this study, may induce artifact formation, or result in the higher abundance of some VOC (Mariaca and Bosset 1997). Dursun et al. (2017) used HS-SPME with 50/30 µm DVB/CAR/PDMS fibre at 55°C for 30 min to extract VOC from UHT milk to determine the correlations between individual aroma VOC and flavour attributes in UHT milks stored at the same conditions. A total of 43 VOCs (aldehydes, alcohols, ketones, acids, aromatic hydrocarbons, nitrogenous, sulphur containing compounds, and an alkane hydrocarbon) were identified. The temperature of extraction (55°C) may again have resulted in increased abundance of some VOC or even artifact formation.

The bipolar CAR/PDMS has also been used extensively to extract VOCs from many dairy products, and it is particularly sensitive for the extraction of low-molecular-weight polar/apolar analytes (up until C6–C8), because of its porosity and the characteristics of its micropores (Mondello et al. 2005; Shirey 2000). The DVB-coated fibres contain relatively few micropores and CAR-coated ones contain a wide range of pores (micro-, meso-, and macro-) in similar volumes (Elmore et al. 2000). Studies have shown that CAR/PDMS is very effective for the analysis of lower boiling

point VOCs (Januszkiewicz et al. 2008; Elmore et al. 2000). Salum et al. (2017) optimized and compared the efficiency of DVB/CAR/PDMS and CAR/PDMS fibres for the extraction of specific VOC (3-methyl-1-butanol, ethyl lactate, 2-nonanone, ethyl octanoate, 2-ethyl-1-hexanol, butanoic acid, phenethyl alcohol, phenol, δ-decalactone, and decanoic acid) in white-brined cheese. These authors found that optimum conditions for the CAR/PDMS fibre were 56.2°C for 84.92 min, slightly different to that for the DVB/CAR/PDMS fibre at 54.75°C for 85.60 min.

Other studies have shown that the CAR/PDMS fiber was more suitable for the extraction of low molecular weight VOCs such as 3-methyl butan-1-ol, ethyl lactate, and butanoic acid) and increasing the extraction time resulted in an increase in the volume of extracted VOCs for CAR/PDMS (Trujillo-Rodríguez et al. 2014). Martin et al. (2011) investigated the effect of oxidoreduction potential (Eh) on the biosynthesis of aroma compounds in non-fat yoghurt by HS-SPME using a 75-μm CAR/PDMS fibre for 40 min extraction time at 50°C. These authors demonstrated that the CAR/PDMS fibre was very sensitive for the extraction of acetaldehyde, dimethyl sulphide, 2,3-butanedione and 2,3-pentanedione. Su et al. (2017) also used the CAR/PDMS fibre to evaluate the VOC profile in yoghurt for 30 min at 60 °C. They identified 30 VOC mainly consisting of aldehydes, ketones and acids plus some alcohols and esters, however the increased temperature of extraction may have inadvertently enhanced the abundance of some VOC or even created new ones. Panseri et al. (2011) developed and validated a HS-SPME GC-MS method to quantify hexanal in butter to monitor lipid oxidation. They used an 85 μm CAR/PDMS fibre at 4 °C for 180 min, the low temperature was selected to minimise matrix oxidation and hexanal production during sampling. The results showed that CAR/PDMS fibre was especially

sensitive to small molecules and suitable to monitor hexanal content both in fresh and oxidised butter samples.

SPME-Arrow has been developed to overcome the capacity limitation of traditional SPME as it has 6 to 20 times more volume capacity (Kim et al. 2020), but is also much less fragile. Manousi et al. (2020) compared a range of traditional SPME fibres (PDMS 100 μm), CAR/PDMS (75 μm), DVB/PDMS (65 μm) to SPME Arrow fibres (PDMS 100 μm), CAR/PDMS (120 μm) and DVB/PDMS (120 μm). These authors found that using CAR/PDMS SPME–Arrow out preformed their equivalent traditional fibre type by 4 or 5 times in terms of recovery using optimised conditions of 50°C for 60 min without salting out for 5mL milk, but it was VOC dependent. However, to date very little studies have been published on SPME-Arrow on dairy products. In addition another new SPME technique, thin film solid phase microextraction (TF-SPME) has been developed that has a very different geometry (a flat planar surface), that effectively increases the surface area-to-volume ratio and thus avoiding the usual caveats of increased phase volume (Bruheim et al. 2003). The simultaneous increase of extraction phase volume and surface area for TF-SPME (CAR/PDMS and DVB/PDMS) devices increases the potential for enhanced sensitivity with as good or better extraction rates compared to traditional SPME fibre (PDMS/DVB) (Emmons et al. 2019), however to date no studies in relation to dairy products appear to have been published.

1.3.3 Stir Bar Sorptive Extraction

Stir bar sorption extraction (SBSE) is another virtually solventless sample extraction technique available with two coatings (PDMS, and PDMS with polyethylene glycol-modified silicone) of varying thickness (Ochiai et al. 2013).

SBSE uses a small magnetic stir bar encased in glass and coated in sorbent material to detect the organic compounds. The principle of SBSE is based on the sorption of VOCs in an aqueous solution or semi-liquid matrix. A major advantage of SBSE is its high sensitivity towards semi-volatiles (Jeleń et al. 2012). The most widely used sorptive extraction phase is PDMS. The choice of extraction coating is a key factor that determines the extraction performance, in terms of extraction efficiency, selectivity and dynamics. PDMS is a commonly used coating for SBSE and it has a good adsorption performance for analytes with weak polarity through hydrophobic force (Fan et al, 2020). The amount of coating (PDMS) in SBSE is usually 50–250 times larger than traditional SPME with 1 cm length × 0.5 mm or 2cm× 1 mm length film thickness, which increases the pre-concentration efficiency (Prieto et al. 2010). The PDMS coating on the stir bar acts as an immobilized liquid into which apolar analytes in an aqueous matrix can partition. The polar matrix components (including inorganic salts, carbohydrates, ionized acids, and amines) do not partition well into the PDMS because of the apolar nature of the PDMS (Baltussen et al. 1999) that significantly aids it performance in extracting VOC as sample component interferences are greatly reduced. After sampling, the extracted analytes are recovered by thermal or liquid desorption and transferred respectively to a GCMS system for analysis. Hoffmann and Heiden (2000) identified different VOCs in milk, condensed milk, cream cheese and yoghurt samples by SBSE coated with PDMS for 60 min at 30 °C. The main VOCs detected were ketones, long-chain FFAs (C:10-C:16), lactones and sulphur compounds. Schiano et al. (2019) compared SBSE (PDMS), HS-SPME (DVB/CAR/PDMS) and solvent assisted SBSE (SA-SBSE) (PDMS) to extract vitamin degradation VOCs from fluid skim milk. The extraction conditions involved submersing the stir bar in cyclohexane for 30 min at room temperature, drying then

adding to milk at 25 °C for 60 min. The results showed that SA-SBSE outperformed both SBSE and HS-SPME in terms of linearity, relative standard deviation and LOD and LOQ. High et al. (2019) compared SBSE, to SAFE, HS-SPME and HS sorptive extraction (HSSE) on reconstituted spray-dried sheep milk. The authors prepared sheep's milk powder in deionized water to 20% solids (w/w). The sample preparation for SBSE (PDMS) involved immersion at 35°C for 90 min and similar conditions for HSSE analysis. For HS-SPME the reconstituted sheep milk was extracted for 60 min at 35°C SPME using the 50/30 μm DVB/CAR/PDMS fibre. For SAFE analysis 250 g reconstituted sheep milk was mixed with 100 mL of dichloromethane and distilled in the SAFE apparatus over a period of approximately 3.5 hr. The organic layer was collected and dried with Na2SO4 (anhydrous) at room temperature under a stream of nitrogen at 100 mL/min. The authors found that SBSE was the most effective technique, with good selectivity, sensitivity, and reproducibility from small sample volumes, although as anticipated some VOC selectivity exists for each technique. Typically extraction times for SBSE are longer than HS-SPME due to the enhanced phase volume, as additional time is required to enable the VOC interact with the phase.

1.3.4 High Capacity Sorptive Extraction

A new high capacity passive SE technique called HiSorb (Markes International Ltd., Bridgend, UK) has been developed that is somewhat similar to SBSE, but more automatable and can also be performed as a headspace (HS) or as a direct immersion (DI) technique (Lancas et al. 2009). Cheng et al. (2021) compared DI-HiSorb (PDMS), HS-HiSorb (PDMS), TD (Tenax/Carbograph) and HS-SPME (DVB/CAR/PDMS) for the extraction of VOCs from WMP, which was reconstituted at 10% solids in ultra-pure deionized water overnight at 4°C prior to evaluation. These authors found DI-HiSorb using a non-polar GC column identified more aldehydes, ketones, lactones,

esters and terpenes than HS-SPME at 40°C for 120 min. These authors also found that DI-HiSorb was particularly effective in extracting lactones in comparison to all the other extraction techniques. Faulkner et al. (2018) compared the efficiency of HS-SPME (DVB/CAR/PDMS) and DI-HiSorb (PDMS) for the extraction of VOCs in pasteurized milk samples. These authors found that an extraction/ equilibration time of 60 min at 37 °C by DI-HiSorb method achieved good results for pasteurized milk samples and identified 38 VOC from a range of different chemical classes, slightly more than the 36 VOC extracted by HS-SPME. Some lactones (γ-crotonolactone, σ-valerolactone, σ-decalactone, σ-dodecalactone) and p-cresol were only identified using DI-HiSorb. Clarke et al. (2022) also used DI-HiSorb (PDMS) at 40°C for 1 hr to extract volatiles in raw milk and managed to successfully identify 99 VOCs consisting of acids (20), alcohols (17), aldehydes (16), esters & ethers (9), furans (3), hydrocarbons & benzenes (7), ketones (10), lactones (5), pyrazines & pyridines (4), sulphur VOC (3) and others (5).

1.3.5 Solvent-Assisted Flavour Evaporation

Solvent-assisted flavour evaporation (SAFE) is an extraction technique which allows the separation and concentration of volatiles by vacuum distillation. SAFE has been shown to extract a great number of aroma compounds from different chemical classes in food (Huang et al. 2019; Zhou et al. 2019). The SAFE distillation system consists of a vacuum pump and usually two cooling traps of liquid nitrogen. The sample is mixed with a solvent (usually diethyl ether or dichloromethane), and the VOCs are collected by distillation with the solvent in the first trap, while impurities and the water condense in the second trap. Engel et al. (1999) provided an overview of the procedure, where they undertook distillation for 36 – 240 min under vacuum (104-106 Pa) at 40-70°C using a circulation water bath. After distillation, the sample

was concentrated under a stream of nitrogen and transferred to a screw-top glass tube for phase separation. SAFE enables the extraction of VOC without extensive preparation, however it is time consuming and expensive due to the requirements for specialist glassware. It is often frequently associated with GC-O analysis, due to the preservation of the heat labile volatiles and lack of artifacts created through extraction at low temperature (Sonmezdag 2019; Whetstine et al. 2006; Evans et al. 2010). However, distillation-extraction techniques are becoming less favourable due to the volumes of solvents required, the time required and the variable recovery rate of highly volatile compounds (Jeleń et al. 2012).

Ning et al. (2011) compared SAFE, SDE and HS-SPME (75 µm CAR/PDMS, 65 µm PDMS/DVB and 50/30 µm DVB/CAR/PDMS) to detect VOC of fermented camel milk. A total of 26 aroma-active VOC were detected by GC-O by SAFE with dichloromethane (20 ml) at 60°C for 30 min. Compared with other pre-treatment methods, the results from SAFE proved to be effective for less volatile and more polar components (mainly alcohols and esters), but also extracted many low boiling points components such as acetaldehyde, ethanol and ethyl acetate. Smith et al. (2016) characterized the VOC profile of milk protein concentrates (MPC 70, 80, 85), milk protein isolates (MPI), acid casein, rennet casein, and micellar casein concentrate (MCC) by SAFE and HS-SPME. The caseins, MPC/MPI, and MCC powders were reconstituted to 10% (wt/vol) in a sodium chloride solution and extraction was performed for 30 min at 40°C by HS-SPME with DVB/CAR/PDMS fibre. The 30 mL reconstituted powder sample was mixed with 100 mL of diethyl ether and SAFE extraction carried out for 40 min at 50°C. The extracts were concentrated under a stream of nitrogen to 20 mL. The VOCs were extracted by HS-SPME and by SAFE. SAFE detected 24 VOC not detected by HS-SPME, and HS-SPME detected 30

compounds not detected by solvent extraction (SAFE). These results highlighted that SAFE tends to favour the extraction of higher molecular weight VOCs. Evans et al. (2009) also used HS-SPME (DVB/CAR/PDMS) and SAFE to extract VOCs in milk serum protein concentrates and in whey protein concentrates (reconstituted at 10% solids, with 10% NaCl). These results demonstrated that SAFE (with 15ml ethyl ether solvent) recovered different classes of VOCs compared with HS-SPME (DVB/CAR/PDMS) at 40°C for 25 min. Mahajan et al. (2004) investigated aroma compounds in sweet whey powder. One kg of sweet whey powder was isolated by solvent (500 mL of 2:1 freshly distilled pentane and diethyl ether solution) extraction followed by SAFE. The most aroma-intense compounds detected by SAFE were short-chain fatty acids, aldehydes and ketones, lactones, sulphur compounds, phenols, indoles, pyrazines, furans, and pyrroles. As mentioned previously High et al. (2019) compared SAFE to HS-SPME (DVB/CAR/PDMS), HSSE (PDMS) and SBSE (PDMS) for the extraction of VOCs in spray-dried sheep milk. These authors found that SAFE was the only extraction technique capable of extracting high concentrations of both the small polar sulphur compounds (dimethyl sulfone) and also larger less volatile lactones. The diethyl ether and dichloromethane solvent was investigated in their preliminary experiment and only dichloromethane was selected for the sheep milk SAFE extraction. This study confirmed that SAFE is suitable to extract highly polar and higher molecular weight VOCs, but is dependent upon the solvent employed. However, the authors found that SAFE was the least reproducible and the least efficient of the methods evaluated. Miyaji et al. (2021) employed SAFE (100g yoghurt samples with 100 mL dichloromethane stirred at room temperature for 1h) to investigate off-flavours from pasteurized drinking yoghurt made from skim milk during long-term ambient storage. Seventy eight VOC were identified. The results

demonstrated that SAFE is very useful in extracting highly volatile compounds which are representative of yoghurt. Lozano et al. (2007) compared DHA (10g butter were purging of the headspace volatiles onto a Tenax TA adsorbent tube by nitrogen at 40°C for 25 min) to SAFE (112g butter combined with 440ml diethyl ether at 30°C for 30 min) to analyse aroma compounds in commercial sweet cream butter by GC-O. A total of 32 and 27 aroma-active compounds were identified by SAFE and DHA, respectively. Some highly volatile compounds such as dimethyl sulphide were lost during workup and concentration using SAFE however, less volatile compounds such as lactones were better recovered by SAFE than DHA. Sarhir et al. (2021) investiaged the VOC profile of Moroccan fermented-salted ‘Smen’ butter and compared purge-and-trap extraction (PTE) at 36°C for 15min with Lichrolut EN (200 mg) sorbent to SAFE (30g butter sample with 80 mL of diethyl ether solvent) at 40°C for 30 min . A total of 27 and 30 aroma compounds were identified by the PTE and SAFE, respectively, but significant differences in the VOCs extracted existed between both methods. The results demonstrated that SAFE was more efficient in the extraction of carboxylic acids than PTE, and the aroma-active compounds detected using SAFE had higher flavour dilution (FD) factors demonstrating that greater concentrations were extracted.

1.3.6 Dynamic Extraction (DE)

In dynamic methods, such as purge and trap (P&T) and TD, the dairy sample is typically heated and the VOCs continuously removed and subsequently concentrated in a cold trap, or adsorbed onto an inert support prior to injection onto the GC. Valero et al. (1997) described the general process used in TD, where VOC are trapped into TD tubes using an inert gas such as nitrogen or helium. In their study, tubes were subsequently desorbed to cold trap to aid peak focussing prior to desorption

to the GC. A wide range of absorbent and adsorbent trapping materials are available and flows can be controlled to split extracts that gives a lot of possibilities to enrich or dilute extracts with relative ease. Samples amounts can be relatively large as the loading capacity of the tubes are large which is beneficial for trace analyte detection (Valero et al. 1997). Cheng et al. (2021) evaluated WMP using TD and had additional equipment such as a Micro-Chamber/Thermal Extractor (μ-CTE) (Markes International Ltd, Bridgend, UK) that provided greater control in the process. These authors used a Tenax/Carbograph sorbent and found that TD was more effective at extracting aldehydes, ketones, alcohols and benzene/phenols, but ineffective for lactones. However some VOC which were not extracted by DI-HiSorb, HS-HiSorb, or HS-SPME but were detected by TD (longifolene, α-terpineol, 1-nananol, p-xylene, 2,3-pentanedione).

P&T is a good technique for the detection of highly volatile compounds with lower boiling points, such as alcoholic compounds and is solvent-free (Mallia et al. 2005), but has generally been surpassed by more automatic extraction methods. Using the P&T technique, the dairy sample is usually homogenized with water, placed in a U-shaped glass sparger and heated. Subsequently an inert gas (nitrogen or helium) is purged through the sample to transfer the VOCs to an inert support of trapping material, which is thermally desorbed and concentrated once again in a cold trap (cryofocusing) before injection onto the GC-column. A wide range of trapping materials are available. Contarini and Povolo (2002) compared to P&T (at room temperature for 60 min with a Tenax trap) and HS-SPME (at 45°C for 30 min with DVB/CAR/PDMS fibre). Both P&T and HS-SPME were comparable in terms of repeatability. The results demonstrated that 11 VOCs were obtained from the milk samples by both PT and HS-SPME. The P&T technique was also better able to extract

smaller molecular weight VOCs (such as, acetone and 2-butanone). Naudé et al. (2009) developed a novel P&T sampling method to extract VOCs from long life UHT milk (2% milk fat) by trapping it on a multi-channel open tubular traps of PDMS for at 45°C for 35 min in nitrogen at 25 ml min^{-1}. The VOCs were subsequently desorbed from the cold traps to the GC using a TD type system. The authors found that dimethyl sulphide, 2-methylpropanal, 2,3-butanedione, 3-methylbutanal, 2-hexanone, 2-heptanone, 2-nonanone, nonanal, and decanal were the predominant VOCs in these samples. Francesca et al. (2015) exploited the potential applications and setup conditions of the automated Gerstel TD (Gerstel GmbH & Co, Mülheim, Germany) using microporus sinthered glass (TDU-CIS4–GC–MS) and cryogenic trapping for the identification of oxidized or non-oxidized volatile compounds of powdered milk at 30 °C for 30 min. They identified 17 VOC mainly consisting of aldehydes, ketones, acids and alcohols. Ciccioli et al. (2004) developed a multiple dynamic headspace extraction TD system for the accurate determination of VOC in goat milk samples. The authors used a series of different traps in an attempt to obtain as true a volatile profile as possible (Tenax, and different types of Carbograph) where helium was passed through the sample at 200 ml min^{-1} at 50 °C to dynamically extract the VOC onto the tubes. The authors also used a colder empty trap before the packed traps in an attempt to reduce moisture getting onto the packed traps. These authors identified 33 VOC mainly consisting of aldehydes, ketones, terpenes, alcohols and benzene compounds in goat's milk.

1.4 Volatiles-Milk, Dairy powders, Butter and Yoghurt

1.4.1 Key Volatiles Associated with the Aroma of Milk and Dairy Powders

VOC including aldehydes, ketones, alcohols, lactones, phenols and esters in milk products originate from the degradation of the major milk constituents (lactose,

citrate, milk lipids and milk proteins) (Cadwallader and Singh 2009), but many are also created through rumen metabolism can also be directly transferred from diet (Kilcawley et al. 2018; Clarke et al. 2022).

Typically the most abundant VOC chemical class in many dairy products are short chain carboxylic acids, known to be major components responsible for the sour taste (Villeneuve et al. 2013; Coppa et al. 2011) and in some cases rancidity (Kilcawley et al. 2018). There are derived from various sources and pathways; lipolysis, carbohydrate metabolism or amino acid metabolism depending upon the specific carboxylic acid (Kilcawley et al. 2018). Simple acids (< 6 carbon) have high odour thresholds, while long chain acids (12 or more carbons) are odourless. Unsaturated acids generally have sharper and stronger odours than saturated ones (Jeleń et al. 2012). A recent study by Clarke et al. (2022) found that butanoic acid (cheesy, dairy, buttery) was a major contributor to the aroma of raw cow's milk. Karagül-Yüceer et al. (2001) determined that butanoic (cheesy), hexanoic acids (cheesy), octanoic acid (waxy, soapy) and dodecanoic acids (fat, sweet) were detected at high odour intensities in the acidic fraction of nonfat dry milk. Karagül-Yüceer et al. (2002) also found that octanoic, nonanoic, decanoic, and dodecanoic acids were associated with soapy/waxy/rubbery attributes in stored nonfat dry milk. These authors also found that octanoic and decanoic acids had very high FD factors and that sour taste was correlated with pentanoic acid. Moreover, propionic acid, 2-methylpropionic acid, 2-/3-methylbutanoic, and pentanoic acids with sweaty or Swiss cheese-like aroma notes were present in the acidic fractions of these nonfat dry milks.

Primary aldehydes are mainly derived from oxidation of polyunsaturated fatty acids (PUFA), but can also be transferred from plant material into milk (Kilcawley et al. 2018; Clarke et al. 2022). The impact of oxidation on VOC generation in milk and

in many dairy products is significant, as the fatty acid profile of milk, especially PUFA is greatly impacted by diet (O'Callaghan et al. 2019). The chain length of aldehydes mostly affects odour thresholds and odour properties. Aldehydes with low molecular weights (< 150 Da) tend to be associated with unpleasant odours, and those with higher molecular weights tend to have sweet, fruity odours (Giri et al. 2010). Milk produced from the cows fed pasture (perennial ryegrass or perennial ryegrass and white clover) was higher in linolenic acid content, which is known to influence the degree of lipid oxidation (O'Callaghan et al. 2016). Havemose et al. (2006) found the level of other primary aldehydes such as hexanal, heptanal, and pentanal increased in milk produced from cows fed grass/clover silage after exposure to fluorescent light compared to milk produced from a hay diet. Feeding pasture has also been shown to significantly elevate the levels of 2-nonenal, hexanal and octanal in milk (Glover et al. 2012). Pentanal is a product of the autoxidation of arachidonic and linoleic acid and was found at greater intensities in milk from cows fed pasture and silage than in milk from cows fed just hay (Villeneuve et al. 2013; Clarke et al., 2020a). Pentanal has also been associated with the cardboard-like or metallic-like off-flavours in milk after prolonged exposure to light (Zardin et al. 2016). Francesca et al. (2015) associated pentanal, hexanal, octanal, 2-heptenal, nonanal, 2-octenal, 2-nonenal, 2-decenal, 2,4-nonadienal, 2-undecenal, 2,4-decadienal with oxidation in powdered milk, defined as 'pungent', 'green (or herbaceous)', 'fat' and 'food-fried'. Boltar et al. (2015) noted that the primary aldehydes nonanal and octanal (products of lipid-oxidation) were significantly higher in milk produced from winter grass silage also highlighting an impact of diet on lipid oxidation. Coppa et al. (2011) found higher benzeneacetaldehyde concentrations in milk from cows on rotational grazing than in milk from a hay-based diet, or from cows on continuous grazing.

Benzeneacetaldehyde is primarily derived from phenylalanine metabolism, but may also be transferred directly into the diet (Coppa et al. 2011; Kilcawley et al. 2018; Clarke et al. 2021). The Strecker aldehydes 2- and 3-methylbutanal (grassy, fatty, astringency, painty) were found to be more abundant in WMP produced from milk of cows fed hay than cows fed diets of maize silage or grass silage and results from the metabolism of isoleucine and leucine, but are also involved in the Maillard reaction (Lloyd et al. 2009).

Ketones are also mainly derived from oxidation of FA in dairy products, but some are also the result of carbohydrate metabolism, it has been suggested that many may not have a significant impact on milk flavour due to their relatively higher odour thresholds and relatively low concentration (Kilcawley et al. 2018). In heat-treated milk, ketones are mainly products of the heat-initiated decarboxylation of β-oxidized saturated fatty acids or decarboxylation of β-ketoacids (Jansson et al. 2014). Contarini et al. (1997) noted that ketones having a higher carbon number are responsible for heated milk flavour. These authors demonstrated that the abundance of 2-heptanone and 2-pentanone increased in milks stored at room temperature and were responsible for heated milk flavour. Moreover, acetone and 2-butanone were also lower in UHT milk and are thought to derive mainly directly from cow's diet (Contarini et al. 1997). Coppa et al. (2011) found that 2,3-octanedione was more abundant in milk derived from diverse pastures and suggested this was due to oxidation of linoleic acid and linolenic acid. Clarke et al. (2020b) found that 3-octen-2-one was correlated with 'caramelised flavour' and 'sweet taste' in WMP. Vazquez-Landaverde et al. (2005) noted that 2-pentanone, 2-hexanone, 2-heptanone, 2-nonanone, and 2-undecanone have been identified as thermally derived off-flavours linked to the level of fat in the milk. Clarke et al. (2020b) found that 3,5-(E,E)-octadien-2-one (grassy, fruity, green),

a product of linolenic acid oxidation, was significantly higher in pasteurised milk derived from concentrate, and correlated with hay-like flavour. Clarke et al. (2022) found that 2,3-butanedione a product of pyruvate metabolism (fresh, sweet, caramel, butterscotch, biscuit, baked) was a key odourant of milk from cows outdoors on pasture (perennial ryegrass).

Sulphur volatiles are potentially very important aroma compounds due to their high odour activities (Falchero et al. 2010). Kobayashi et al. (2008) found methyl 2-methyl-3-furyl disulphide, furfuryl methyl disulphide, and bis(2-methyl-3-furyl) disulphide were present in high-heat treated SMP and in UHT milk, which presented a ‘canned corn-like’, ‘rice bran-like’, and ‘vitamin-like’ odour profile. Vazquez-Landaverde et al. (2005) found that dimethyl sulphide was almost three times higher in UHT than in raw milk, and was formed from the sulfhydryl group of milk proteins subjected to thermal denaturation. Clarke et al. (2022) found that methanethiol (cabbage) was an important odorant in raw cow’s milk from pasture (perennial ryegrass).

Terpenes are naturally occurring plant secondary metabolites derived from isoprene units (C5) and also derived from larger terpenoids; monoterpenes (C10) and sesquiterpenes (C15). Terpenes are odour active but have a high odour threshold and therefore need to be at high concentrations to have a sensory impact (Kalač 2011). Ciccioli et al. (2004) noted the maximum monoterpene (α- and β-pinenes) content in milk was associated when the largest variety of herbs was present in the pasture. Faulkner et al. (2018) also found that β-pinene is most likely derived directly from forage, but concentrations are dependent upon the diversity of the pasture. These authors also found that β-pinene was absent in cow’s milk derived from a concentrate diet. Coppa et al. (2011) found the concentrations of β-pinene and cymene-(p) and all

sesquiterpenes (β-caryophyllene, alloaromadendrene, germacrene-D, and γ-cadinene) were higher in milk derived from animals on continuous grazing than on less diversified pasture under rotational grazing. Coppa et al. (2011) also found that sesquiterpenes were more influenced by different grazing systems than monoterpenes. Limonene (sweet citrus–like) is also a product of bioconversion of sesquiterpenes and was the most common terpene in milk from a range of highland and lowland pastures (rye-grass, clover) or from concentrates (maize silage, hay, cereals) over different seasons (Fernandez et al. 2003).

Phenolic compounds can be important volatile compounds in milk related to forage intake. Alkylphenols in ruminant milks are derived from phenolic compounds ingested through feed and were responsible for the 'cowy flavour' of milk (Feo et al. 2006). p-Cresol is a major alkylphenol and has a characteristic 'barn-like flavour' that blends with the more medicinal notes of m-cresol in milk (Ha and Lindsay 1991). Faulkner et al. (2018) found a direct link between p-cresol levels in raw milk from cows fed clover with 'barnyard aroma', which was also subsequently linked to isoflavone metabolism by Clarke et al. (2019). Karagül-Yüceer et al. (2002) noted that p-cresol and skatole may be the contributors to undesirable flavours in milk. Phenols (clove-like, medicinal, smoky) are described as heat-generated compounds in UHT milk (Dursun et al. 2017). Most phenolic compound are excreted, but some end up in milk and depending upon their abundance may influence sensory perception.

Hydrocarbons compounds with high odour thresholds can also play an essential role in food aroma when present at high concentrations (Czerny et al. 2011). Toluene, is a product of β-carotene light-induced oxidation, has been implicated as responsible for rancid notes and was more abundant in pasture-derived milk than milk

from cows fed indoors (Coppa et al. 2011). Faulkner et al. (2018) found toluene was significantly higher in CLV milk than concentrate milk and linked as a potential biomarker for pasture, derived from metabolism in the rumen. Xylene (sweet) may be the result of carotenoid degradation, namely β-carotene degradation in the rumen or possibly directly transferred from feed (Buchin et al. 1998).

Lactones are cyclic compounds formed by the intramolecular esterification of hydroxyacids through the loss of water, described as having a buttery-type, creamy, fruity or otherwise pleasant odour. Few differences in lactone content were linked to diet but they appear to be more important in pasteurized milk than in raw milk because heat is a factor in their production (Urbach 1997; Li et al. 2020). Villeneuve et al. (2013) found the detection intensity of δ-octalactone and δ-tetradecalactone were affected by forage types. In their study, the content of γ-decalactone, γ-dodecalactone, and γ-dodecaenolactone in milk were higher in hay-fed cows, lower in silage-fed cows, and intermediate for cows on pasture. Karagül-Yüceer et al. (2002) showed that δ-decalactone and γ-dodecalactone gave sweet odour properties to milk powder. Sweet and milky odour properties were characterized by lactones including γ-undecalactone, γ-dodecalactone, δ-decalactone, and δ-undecalactone. Clarke et al. (2022) found that γ-butyrolactone was an important odour active volatile in raw cow's milk, and that γ-hexalactone influenced the aroma of cow's milk produced from a concentrate diet.

1.4.2 Key Volatiles Associated with the Aroma of Butter

Garvey et al. (2020) found pentanal (paint-like) and decanal (green, fatty), derived from oleic acid and linoleic acid (also arachidonic acid for pentanal), were more abundant in butter produced from cows outdoors fed perennial ryegrass and white clover than perennial ryegrass alone or from cows indoors fed concentrate. In

their study, heptanal was significantly more abundant in butters produced from milk derived from a pasture (perennial ryegrass, or perennial ryegrass and white clover) in comparison to a concentrate diet, and has a ‘green sweet’ aroma. Glover et al. (2012) noted butanoic acid levels were higher in butter produced from milk from cows fed the concentrate compared with pasture. Butanoic acid is likely a very important aroma compound in butter and was a main contributor to ‘fresh butter’ aroma in sweet cream butter (Lozano et al. 2007). O’Callaghan et al. (2016) found acetone (earthy, strong fruity, hay) was significantly correlated with butter produced from milk derived from cows outdoors on perennial ryegrass and white clover diets, than in butter produced from milk from cows outdoors on perennial ryegrass, or indoors on concentrate. These same authors also found 2-butanone (buttery, sour milk, etheric) was significantly more abundant in butter produced from concentrate diets. Mallia et al. (2008) found that the concentrations of 1-octen-3-one (mushroom) increased when butter oil was stored at room temperature. Li et al. (2020) found 3-penten-2-one is a product of lipid oxidation, with low levels indicating freshness in butter. 2,3-Butanedione is a very odour-active compound with a characteristic buttery aroma, derived from pyruvate (Liu et al. 2020b). Garvey et al. (2020) found 2,3-butanedione was significantly more abundant in butter produced from milk derived from cows outdoors fed perennial ryegrass and white clover compared to butter produced from cows indoors on a concentrate diet. Li et al. (2020) found that δ-decalactone was the most important odour active aroma in butter, and that overall lactones in general were important odour compounds in butter. Lozano et al. (2007) compared the VOC profile of fresh sweet cream butter and butters stored at refrigeration (4 °C), frozen (-20 °C) and at room temperature. These authors identified butanoic acid, δ-octalactone, δ-decalactone, 1-octen-3-one, 2-acetyl-1-pyrroline, dimethyl trisulphide and 2,3-

butanedione as the most intense aroma compounds associated with fresh butter samples and that dimethyl sulphide is possibly a contributor to cooked/ nutty flavour in butter, which is in agreement with Contarini et al. (2002). Lozano et al. (2007) also noted that the main changes in aroma active VOC over storage was related to an increase in the intensity of lactones (δ-octalactone, δ-decalactone and δ-dodecalactone), lipid oxidation VOC ((E)-2-nonenal, 2-heptanone, (Z)-4-heptenal, (E,Z)-2,6-nonadienal and hexanal) and acidic odourants such as acetic and butanoic acids. These authors also noted that styrene levels increased over storage due to migration from packaging material, and may adversely impact on fresh butter flavour.

Lazono et al. (2007) also suggested that toluene (nutty, bitter, almond, plastic) may be associated with stale butter flavour it is a product of β-carotene degradation and has been previously shown to be significantly higher in butter derived from milk of cows fed outdoors on pasture (perennial ryegrass or perennial ryegrass and white clover) than cows indoors fed concentrate (O'Callaghan et al. 2016).

1.4.3 Key Volatiles Associated with the Aroma of Yoghurt

Acetaldehyde, predominantly derived from pyruvate decarboxylation or generated by the metabolism of threonine, is a major aroma compound in yoghurt and exhibits a green apple or nutty flavour (Settachaimongkon et al. 2014; Cheng et al. 2010; Eram and Ma. 2013). Tian et al. (2017) demonstrated that the concentration of acetaldehyde increased after the end of fermentation, reached a maximum at the beginning of storage, and then declined sharply with increasing storage time. This study highlighted that yoghurt samples fermented with a *Lactobacillus acidophilus* culture produced the highest concentrations of acetaldehyde in comparison to other strains evaluated. Tian et al. (2019) also demonstrated that acetaldehyde contributes a

‘green apple’ or ‘nutty’ attribute at lower concentrations, but negatively influences aroma at high concentrations. 2,3-Butanedione and acetoin are produced by pyruvate or citrate metabolism by various lactic acid bacteria, and are typical carbonyl compounds and contribute greatly to the ‘butter and cream’ aroma of yoghurt (Hugenholtz, 1993; Neves et al. 2005). Acetoin, derives from the enzymatic degradation of 2,3-butanedione and although has a much weaker aroma than 2,3-butanedione helps to contribute to a ‘mild creamy’ aroma in yoghurt (Cheng et al. 2010). Innocente et al. (2016) found that a 1:1 acetaldehyde to 2,3-butanedione ratio gave the most preferential yoghurt aroma, while too much acetaldehyde resulted in a ‘green off-flavour’. A study by Tian et al. (2017) demonstrated that 2,3-butanedione and acetoin reached maximum concentrations after 14 d refrigerated storage post production. Acetoin, 2,3-butanedione and 3-heptanone are also all known to contribute to yoghurt odour by providing ‘fruity, sweet’ aromas (McSweeney and Sousa 2000; Gallardo-Escamilla et al. 2005). Tian et al. (2017) reported that 2-butanone, 2-pentanone and 2-heptanone (originating from oxidation, carbohydrate metabolism and/ or direct transfer) were all identified as significant aromatic volatiles in yoghurt samples fermented with *Lactobacillus casei*. Short-chain fatty acids are also produced during yoghurt fermentation by both lipolytic processes and by lactic acid starter fermentation (Tamine and Richard 2007). Acetic acid, one of the important acidic compounds produced by hetero fermentative LAB contributes an undesirable vinegar taste at high concentrations, which can unbalance the overall flavour (Buffa et al. 2004). However, as acetic acid is not that odour active excessive levels are generally not a major issue in yoghurt production. Innocente et al. (2016) demonstrated that both hexanoic and butanoic acid were significantly higher in yoghurt samples fermented with *Lactobacillus casei* than yoghurt fermented with

Lactobacillus rhamnosus. Tian et al. (2019) documented that butanoic and octanoic acids contribute to the characteristic cheese flavour of yoghurt, and that decanoic acid provides a ‘light cream’ flavour. Some alcohol compounds also contribute to the aroma of yoghurt. Lower alcohols (from C1 to C10) affect the flavour of yoghurt, and can be important as they positively influence sensory perception (Cheng 2010). Ethanol, the final product of glucose metabolism or amino acid degradation in milk, is thought to influence sweetness (Urbach 1995), but unlikely to have a major contribution due to its very high odour threshold. Tian et al. (2019) demonstrated that 3-pentanol and 1-hexanol contribute to a ‘grass’ flavour in yoghurt. 1-Pentanol and 2,3-butanediol, were demonstrated to provide a ‘fruit’ flavour and improve the overall flavour quality (Tian et al. 2019).

1.5 Gas Chromatography Olfactometry -Milk, Dairy powders, Butter and Yoghurt

In GC-O the human nose is used as a detector to evaluate the character and odour intensity of VOCs (Zellner et al. 2008). Thus, it is possible to discern key aromatic compounds in dairy products by GC-O, but impossible to completely understand the whole aroma profile using GC-O, partly because other factors influence aroma perception, but also because aromas often consist of a combination of two or more VOC. As previously stated VOCs are challenging to extract, separate, identify and quantify as they can interact synergistically or additively to produce an overall odour (Brattoli et al. 2013). Even though GC-O has existed for decades it remains a relatively obscure research technique especially for milk, dairy powders, butter or yoghurt (~10 publications to date). Overall the limited use of olfactory analysis for these products is difficult to fathom as even though it is not a complete solution in relation to fully understanding the relationship between VOC and aroma perception,

it does provide a very good insight into the aroma characteristics of a product. A likely aspect for its limited use is that it requires highly trained assessors and is quite time consuming (Zellner et al. 2008). However, the potential benefits easily outweigh any disadvantages. GC-O and chemical sensor technologies such as electronic nose and tongue (e-nose and e-tongue), combined with multivariate data processing methods are promising relatively novel approaches for rapid analysis of food (Wardencki et al. 2013). Merging both GC-O and GC–MS, as an integrated instrument is particularly useful for the identification of aroma-active VOC.

To date, HS-SPME, SAFE and dynamic headspace sampling (DHS) are the most commonly used as pre-treatment methods for GC-O analysis (Song and Liu 2018). VOC are typically present from trace amounts to even a few mg kg^{-1} (such as fatty acids in cheese) with odour thresholds varying from ppt to many ppm. In strong smelling dairy products, it may not be necessary to concentrate the VOC profile for GC-O, but for products such as fresh milk it is necessary, thus the choice of extraction method can be dependent upon the sample type. However, care must be taken in GC-O to avoid losing thermal labile VOC or specifically enhancing or creating VOC during the extraction processes, otherwise spurious information may be generated.

Table 3 covers the key VOCs classes associated with milk, milk powder, butter and yoghurt products. A recent study employed HS-SPME-GC-O (8mL milk sample with 2g of sodium chloride at 50◦C for 40 min extraction with 75 μm DVB/CAR/PDMS), to compare the volatile profiles of raw and pasteurized milk and pulsed electric field (PEF) treated milk (Zhang et al. 2011). PEF is a non-thermal processing technology that can be applied to liquid milk to inactivate both spoilage and pathogenic microorganisms but also maintains the original nutrients of milk (Amiali and Smith 2007). In the study by Zhang et al. (2011), a total of 19 active VOC

were detected with aldehydes making major contributions to a ‘fruity, green, cream’ note in both pasteurized and PEF-treated milk. 2(5H)-Furanone was only detected in PEF treated milk and described as ‘caramel’ odour. Although concentrations of aldehydes and methyl ketones differed between pasteurized and PEF-treated milk, it appeared not to impact their aroma activities. Colahan-Sederstrom and Peterson (2005) determined if epicatechin addition to raw milk would inhibit the thermal generation of Maillard-type aroma compounds in UHT-processed fluid milk. A total of 32 aroma-active VOC were identified in UHT milk using SAFE-GC-O and GC-MS (1 kg milk sample was extracted with 875 mL diethyl ether for 1h at 40 °C). Methional, furfural, 2-isopropyl-3-methoxypyrazine, 2-acetyl-1-pyrroline, and 2-acetyl-2-thiazoline (Maillard-type aroma compounds) showed the largest changes in FD post heat-treatment and contributed to the ‘cooked’ and ‘bitterness’ flavour of UHT milk. This study demonstrated that epicatechin had the greatest inhibitory effect on the Maillard-derived compounds. In another study, a direct solvent extraction and high-vacuum distillation extraction method was developed for detection of chemical and sensory profiles of stored nonfat dry milk by GC-O (Karagül-Yüceer et al. 2002). Fifty six aroma active VOCs were detected and a variety of aldehydes, ketones, alcohols, and free fatty acids were found to be responsible for the development of undesirable flavours. These authors stated that p-cresol, 3-methylindole (skatole), and some unknown compounds with ‘cowy’, ‘fecal’ or ‘animal-like’ odours appear to contribute to undesirable flavour in milk. Methional and o-aminoacetophenone had high odour intensities in these nonfat dry milks and had characteristics ‘boiled potato’ and ‘animal’ odours, respectively. Free fatty acids including butanoic and hexanoic (cheesy notes) and octanoic, nonanoic, decanoic, and dodecanoic acids (waxy note), were also found to contribute to the aroma of milk. Sun et al. (2021) investigated key

aroma-active compounds in butter by SAFE-GC-O and GC-MS (40g butter distilled with 200mL dichloromethane at room temperature for 30min). Fifty-three odorants were identified. 2-Furfurylthiol, 2-acetylthiazole, anethole, (E)-2-decenal, and 1,8-cineole were the key odorants for the overall aroma of butter, and contributed the 'beef', 'boiled beef', 'anise', 'tallow', 'mint, herb' aromas, respectively. As previously mentioned Lozano et al. (2007) investigated the major aroma components of sweet cream butter. These authors identified 32 and 27 aroma-active VOC were identified by SAFE-GC-O and DHS –GC-O, respectively. VOCs such as lactones were easily recovered by SAFE but poorly by DHS. Butanoic acid, 1-octen-3-one, 2,3-butandione, 2-acetyl-1-pyrroline, dimethyl trisulphide, δ-octalactone and δ-decalatone were the main contributors to fresh butter aroma. Peterson and Reineccius (2003) determined key odourants in heated sweet cream butter aroma by using static headspace analysis (5.6g butter solution placed in purge and trap vessel for 45min extraction at 38°C by Tenax TA trap) by GC–O. These authors identified 19 odour-active VOC in the HS of heated butter. Methanethiol, methional, 3-methylbutanoic acid, 2-heptanone and furaneol were the key odour-active VOC in heated butter in comparison to fresh butter, and contributed to the 'pungent', 'cooked potato', 'cheesy', 'blue cheese' and 'sweet caramel' aroma, respectively. Liu et al. (2022) evaluated the odour-active VOC of yoghurt using DHS, SPME, SAFE, and SBSE/GC-O and by GC-MS. A total of 31 odour-active VOC were perceived by four extraction methods with DHS providing the most VOCs. 2,3-Butanedione, hexanoic acid, acetophenone, 2,3-pentanedione, acetic acid, octanoic acid, 3-methyl-2-buten-1-ol, butyl acrylate, 2-heptanone, ethyl 2-methylbutyrate, and ethyl butyrate were identified as the key odour-active components of yoghurt by DHDA (Dynamic headspace dilution analysis). Aroma extraction dilution analysis and odour activity value identified 'green apple-like', 'sweat-like',

‘sweet-like’, ‘fruit-like’, ‘butter-like’, ‘vinegar-like’, ‘red bean-like’, ‘green-like’, and ‘cream-like’ flavour properties in these yoghurts. 2,3-Butanedione was found to be the most important odour-active VOC with the highest FD value in yoghurt, contributing ‘buttery’ odour.

Table 1.3 Volatile compounds identified in milk, milk powder, yogurt and butter by GCO-MS.

Compounds	Odour Description	Milk	Milk Powder	Yogurt	Butter	Reference
Alcohols						
Methanethiol	Pungent, sulphury				✓	f
Ethanol	Floral, medicine				✓	g
3-Methyl-1- butanol	Floral-fresh, cheesy, rubber, painty	✓			✓	c, g
2-Heptanol	Fatty-oily				✓	g
2,3-Butanediol	Creamy			✓	✓	g, e
1,3-Butanediol	Musty-wet				✓	g
1-Octen-3-ol	Mushroom, earthy	✓			✓	a, b, h
1-Heptanol	Mushroom				✓	h
Furfuryl alcohol	Caramel				✓	h
α-Terpineol	Green				✓	h
2-Butanol	Sweet almond-like			✓		e
1-Butanol	Balsam-like, burnt, sweet	✓		✓		c, e
1-Pentanol	Sweet			✓		e
1-Hexanol	Greasy			✓		e
2-Ethylhexanol	Citrus			✓		e
2-Phenylethanol	Rose		✓			j
Aldehydes						
2-Methylpropanal	Dark chocolate				✓	i
2/3-Methylbutanal	Dark chocolate, sweet, fruity, fatty	✓	✓	✓	✓	b, i, e, j
Hexanal	Green, grass, tallow, fruity, floral	✓	✓	✓	✓	b, c, d, h, i, e, j
(Z)-4-Heptenal	Rancid, crabby, biscuit-like	✓	✓		✓	b, i, j
Nonanal	Mushroom,waxy,fatty, floral, green, rosy, sweet, floral	✓		✓	✓	d, e, h, i

(continued)

Decenal	Green, fatty, floral			✓	✓	e, h, i
(E)-2-Nonenal	Hay, green, fatty, cucumber, oxidized	✓	✓		✓	a, b, f, i, j
(E,Z)-2,6-Nonadienal	Cucumber, cardboard	✓	✓		✓	a, b, i, j
Acetaldehyde	Green, pungent, apple like			✓	✓	e, f
Benzaldehyde	Almond-nutty			✓	✓	e, g
Heptanal	Fat, citrus, cheesy, caramel, fruity	✓			✓	c, d, h
(E)-2-Hexenal	Apple, green				✓	h
Octanal	Fat, soap , orange, fragrant, citrus	✓		✓	✓	c, d, h, e
(E)-2-Heptenal	Fat, fruity	✓			✓	c, h
(E,E)-2,4-Hexadienal	Fat, green	✓			✓	c, h
(E)-2-Octenal	Green, fatty	✓	✓		✓	b, c, h, j
(E,E)-2,4-Heptadienal	Fat, green	✓			✓	c, h
(E)-2-Decenal	Tallow				✓	h
(E)-2-Undecenal	Fat, metallic	✓			✓	b, h
(E,E)-2,4-Decadienal	Fat, soapy, hay, fried	✓	✓		✓	b, h, j
(E,E)-2,4-Nonadienal	Cardboard	✓	✓			a, j
Butanal	Cocoa-like			✓		e
Pentanal	Fermented like, fruity, floral	✓		✓		d, e
Methional	Cooked potato	✓			✓	a, b, f
Phenylacetaldehyde	Rose		✓			j
Carbonyl compounds						
Ethenylbenzene (styrene)	Styrene, plastic				✓	i
p-Cresol	Cowy, barny	✓	✓			b, j
Toluene	Painty	✓				c
Ketones						
2,3-Butanedione	Buttery, cream, cheese	✓	✓	✓	✓	b, d, e, f, i, j

(continued)

1-Hexen-3-one	Plastic, veggie, rubbery	✓		✓		✓	b, f, i, j
2-Heptanone	Fatty,blue cheese,cheesy-nutty,sweet, fruity, milky, plastic	✓			✓	✓	d, e, f, g, i
Acetoin	Buttery-creamy, mild creamy				✓	✓	e, g
2-Nonanone	Milky, sweet, herb-like				✓	✓	e, g
Acetone	Fruity				✓		e
2-Butanone	Fruity, buttery, cheese		✓		✓		d, e
2-Pentanone	Wine-like, Malty, fruity		✓		✓		d, e
3-Hexanone	Rum-like				✓		e
2,3-Pentanedione	Sweet				✓		e
3-Heptanone	Green				✓		e
2-Undecanone	Fruity				✓		e
Acetophenone	Sweet-almond				✓		e
(Z)-1,5-octadien-3-one	Metallic			✓			j
3-Hydroxy-2-butanone	Buttery, creamy		✓				d
Lactones							
δ-Octalactone	Herbaceous, peach		✓			✓	a, f, i
γ-Nonalactone	Peachy					✓	i
δ-Decalactone	Waxy, sweet, coconut, hot milk		✓	✓		✓	a, b, d, j
δ-Undecalactone	Coconut, butter, green, cilantro		✓	✓		✓	b, i, j
δ-Dodecalactone	Coconut, cheesy, sweet, fruity		✓		✓	✓	d, e, i
γ-Dodecalactone	Sweet, green		✓	✓			b, j
δ -Decanolactone	Peach					✓	f
δ -Hexanolactone	Creamy, chocolate, sweet aromatic					✓	f
γ-Decalactone	Sweet, perfume		✓				a
γ-Butyrolactone	Creamy				✓		e

(continued)

Sulphur compounds						
Dimethyl sulphide	Sulphur, sweet			✓	✓	e, i
Dimethyl trisulphide	Cabbage, garlic, sulphury	✓	✓		✓	b, f, i, j
Ethyl disulphide	Gasoline		✓			j
Hydrogen sulphide	Boiled egg, eggy				✓	f
Dimethyl disulphide	Vegetable-like			✓		e
Esters						
Ethyl acetate	Fruity, mild , sweet, solvent	✓		✓	✓	d, e, i
Ethyl butanoate	Fruity, berry, fruity-rose ,herb-like/sweet			✓	✓	e, g, i
Ethyl lactate	Creamy-whey				✓	g
Ethyl octanoate	Floral				✓	g
Ethyl decanoate	Fruity-pear				✓	g
Ethyl propionate	grape-like			✓		e
Methyl butanoate	pineapple-like			✓		e
Butyl propionate	rosy/sweet			✓		e
Ethyl hexanoate	pineapple-like			✓		e
Furans						
Furaneol	Sweet caramel-like	✓			✓	a, f, h
Furfural	Almond, roasted, nutty	✓			✓	a, c, h
Furfuryl alcohol	vitamin, rubber,caramel	✓	✓		✓	b, h, j
2-Furanmethanol	toast bread-like			✓		e
2-Acetylfuran	Plastic,nutty	✓				c
Acids						
Acetic acid	Vinegar,Sour	✓		✓	✓	c, e, h, i
Butanoic acid	Fecal, cheesy, rancid, ripened cheese, buttery, sour, creamy	✓		✓	✓	d, f, g, i, e

(continued)

3-Methylbutanoic acid	Sweaty, cheesy, whey-flowery, sour	✓			✓	a, b, f, g, i
Hexanoic acid	Doughy, sweaty,cheesy acrid,rancid, buttery-soapy,sour	✓		✓	✓	a, b, d, e, f, g, i
Propanoic acid	Fatty, cheesy			✓	✓	e, g
2-Methylpropanoic acid	Rancid buttery			✓	✓	e, g
Pentanoic acid	Cheesy-musty, swiss cheese				✓	b, g
Octanoic acid	Cheesy, goat, foul	✓		✓	✓	a, g, e
Nonanoic acid	Green, fat, sour	✓		✓	✓	a, g, e
Decanoic acid	Soapy, rot-like	✓		✓		a, e
Butyric acid	Sour	✓				a
Heptanoic acid	Sour	✓		✓		a, e
Dodecanoic acid	Waxy	✓				b
Tetradecanoic acid	Coconut-like			✓		e
Terpenes						
α-Pinene	Mint, pine oil, dry, woody	✓			✓	c, i
D-Limonene	Citrusy, Lemon, orange			✓	✓	g, h, e
β-Myrcene	Balsamic, rosin				✓	h
3-Methylthiophene	Plastic		✓			j
Other						
2-Acetyl-1-pyrroline	Popcorn, roasted	✓	✓		✓	a, b, i, j
2-Acetyl-2-thiazoline	Cooked, popcorn, roasted	✓	✓		✓	a, b, i, j
Skatole	Skatole, fecal, mothball		✓		✓	i, j
Acetylpyrazine	Roast				✓	h
Ethenylbenzene (styrene)	Overripe fruit, clean			✓	✓	e, g
Benzothiazole	Rubber		✓			j
β-ionone	Hay		✓			j

The data adapted from

a Colahan-Sederstrom and Peterson, 2005
b Karagül-Yüceer *et al*. 2001
c Yeh *et al*. 2017
d Zhang *et al.* 2011
e Liu *et al.* 2022
f Peterson and Reineccius, 2003
g Sarhir *et al.* 2021
h Sun *et al.* 2021
i Lozano *et al.* 2007
j Karagül-Yüceer *et al.* 2002

1.6 Conclusions

Significant advances in our understanding of the key aroma active VOC that impact the sensory perception of milk, dairy powders, butter and yoghurt have been outlined. In terms of sensory approaches both traditional and novel sensory techniques have been discussed, but also cultural factors influencing choice. This review has reiterated the importance of product familiarity and how critical this is in relation to cross cultural sensory acceptance, especially in countries where dairy products have little tradition. Much more sensory research of dairy products is required to better understand cultural factors influencing choice/ acceptability, and to ensure that all participants in such studies unambiguously comprehend what is required of them, most notably avoiding words that could be miss-interpreted or have dual meanings from a cultural or language perspective.

The importance of VOCs impacting the aroma of milk, dairy powders, butter and yoghurt are discussed. More than 300 different VOC, belonging to 10 or more chemical classes have been identified in milk, dairy powder, butter and yoghurt to date. This review has focussed on GS-MS which is by far the most widely used approach to identify these compounds, but with particular emphasis on the different VOC extraction techniques used, highlighting their advantages and/ or shortcomings. Aldehydes, alcohols, lactones, ketones, acids, terpenes, carbonyl compounds, and furans are by far the most prominent and potent VOC that appear to influence the sensory appeal of these products. A single or multiple source can be responsible for the generation of VOC. Some are directly or indirectly dietary related, in that they can be transferred from the diet by ingestion or inhalation, or indirectly created during rumen metabolism and end up in the milk. Others are created during processing for example by heat treatments or by the inclusion of ingredients/ processing aids or in

final product formulation. Thus diet and milk quality plus product processing and formulation have a major role in VOC formation in the final product, which subsequently impacts on aroma generation and thus sensory perception.

As product variation within these dairy products is relatively large, and as a wide range of odour active VOC are typically present, it is difficult to absolutely identify individual VOC responsible for the overall aromatic characteristics of these dairy products. However, some informed conclusions can be made based on research to date. This review has highlighted the benefits of GC-O, especially in combination with complementary techniques such as GC-MS and also highlights that much more research is required combining sensory and analytical techniques in order to better understand flavour development in these products in order to improve quality but also adjust in-farm and process inputs to create products more suited to particular markets.

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Chapter 2. A cross-cultural sensory analysis of skim powdered milk produced from pasture and non-pasture diets

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Abstract

Understanding potential cross-cultural sensory differences in the perception of Irish dairy products is important for key markets such as the USA and China. As most Irish dairy products are produced from pasture derived milk, this study investigated the impact of pasture and non-pasture diets on the cross cultural sensory perception of skim milk powder (SMP) in Ireland, USA and China. SMP was produced from cows fed outdoors on ryegrass (GRS), ryegrass/white clover (CLV), and indoors on total mixed rations (TMR). SMP samples were evaluated by Irish (n = 78), USA (n = 100) and Chinese (n = 106) consumers using an identical hedonic sensory acceptance test in Ireland, USA and China. Optimized Descriptive Profiling (ODP) was performed using trained assessors familiar with dairy products in Ireland (n = 25) and China (n = 22), and traditional descriptive analysis was undertaken by a trained panel (n = 7) in the USA. Volatile analysis was undertaken on each SMP sample. Hedonic assessment found that USA consumers preferred SMP derived from TMR, and Irish consumers preferred SMP from either GRS or CLV. Chinese consumers perceived SMP samples differently to the USA and Irish consumers, but preference was not influenced by diet. Both Irish and Chinese trained assessors found it more difficult to discern differences between GRS or CLV SMP, but could differentiate TMR SMP. Irish assessors preferred GRS and CLV SMP. Chinese and Irish assessors had different preferences for many attributes. Trained USA panelists found significant differences, exclusively associating pasture based diets with “cowy/barny” and “cardboard/wet paper” attributes and more intense “grassy/hay” attributes than in TMR SMP. The abundance of ten volatile compounds differed significantly based on diet with acetoin derived from carbohydrate metabolism at much greater abundance in TMR SMP. This study found that sensory perception and volatile profiles of SMP were influenced by diet

and differences in sensory perception existed between the three cultural groups. Irish and USA sensory responses aligned with familiarity of dairy products derived from pasture and non-pasture diets, respectively, and Chinese sensory responses differed to Irish and USA responses likely reflecting their lack of familiarity with dairy products.

Keywords: Cross-cultural sensory, Bovine diet , Skim milk powder, Volatile analysis

2.1 Introduction

There is an increased demand for pasture-fed dairy products resulting from consumer perceptions of a healthier, “added value” and “more natural” products than those derived from indoor feeding systems (Kilcawley et al. 2018). From a nutritional point of view, bovine milk derived from pasture feeding provides better protein, omega-3, and vitamins than from conventional indoor ration feeding systems (Elgersma, 2015; Egan et al. 2018). As the Irish dairy sector is export driven, it is incumbent to have a greater understanding of the factors influencing sensory perception in key global markets. Different food environments and dietary experiences across cultures are known to influence both sensory perception and consumer behaviour (Prescott and Bell, 1995). Therefore, the understanding of food familiarity by consumers and cultural differences associated with food perception is vital in sensory research to understand differences in consumer behaviour (Soares et al. 2017).

The familiarity of food products plays a vital role in the acceptability and preferences of consumers because it delineates cultural differences in food, as consumers tend to favour familiar food (Torrico et al. 2019). Familiarity is acquired through previous experience and repeat exposure which decreases the uncertainty with regard to safety and identity and thus has a positive impact on liking (Methven et al. 2012). A recent study by Kim et al. (2018) demonstrated that familiarity affected consumers’ perception of nuttiness in soymilk amongst Chinese, Korean and Western consumers. In another study of Mexican and Brazilian nopal (Opuntia cacti) consumers, differences in acceptance and perception were found to be mainly due to differences in familiarity rather than natural personal variations (de Albuquerque et al. 2018). Moreover, Kim et al. (2018) reported that French and Korean trained panels

showed different preference patterns according to familiarity especially when evaluating unfamiliar green tea products.

Previous studies have highlighted that the feeding system of dairy cows can affect the composition of milk with pasture derived milk having significantly higher proportions of unsaturated fatty acids (UFA) and conjugated linoleic acid (CLA) compared with those derived from total mixed ration (TMR) diets (Coakley et al. 2007). Feeding systems have also been shown to alter volatile compounds in bovine milk as these can transfer directly, and indirectly through rumen metabolism from forage into milk (Kilcawley et al. 2018; Martin et al. 2005). Volatiles derived from the metabolism of dietary components can also have a sensory impact; "barnyard" aroma/flavor appears to be linked to p-cresol content in milk (Faulkner et al. 2018; Martin et al. 2005). Croissant et al. (2007) documented sensory flavor differences by both trained panelists and consumers between milk from grass fed cows and milk from cows fed a TMR diet. In the sensory evaluation of milk and cheese, differences in visual (mainly color) attributes appear easier to discern than flavor differences by both trained and untrained panels (Kilcawley et al. 2018). The feeding system can also affect the natural color of products, and TMR diets produce dairy products that are whiter in color than those of pasture feeding systems, which have a characteristic yellow color because of the higher carotenoid (β-carotene) content (Hurtaud et al. 2002, O'Callaghan et al. 2016a).

Dairy powders are major commodity export products and this study assessed consumer perceptions of bovine skim milk powder (SMP) from three feeding systems; TMR diet indoors, perennial ryegrass outdoors and perennial ryegrass/white clover outdoors in Ireland, China and USA. Volatile analysis was also undertaken to

determine if sensory attributes could be chemometrically associated with specific volatiles.

2.2 Materials and Methods

2.2.1 Skim Milk Powder Manufacture

In this study SMP was evaluated as it remains a considerable dairy export product for Ireland and has a longer shelf life than whole milk power. Raw milk from 54 Friesian cows was split into three groups (n = 18) at the Teagasc Moorepark dairy farm, Fermoy, Co. Cork, Ireland. Each group of 18 cows were given separate diets, (A) outdoors on perennial rye-grass only (GRS), (B) outdoors on perennial ryegrass/ white clover (CLV) and (C) indoors on TMR as described by O'Callaghan et al. (2016b).

Raw whole milk (approximately 1000 kg) was collected from the cows on each dietary treatment. Milk was pre-heated to 50 ˚C in an APV plate heat exchanger (SPX Flow Technology, Crawley, West Sussex, UK), separated by a centrifugal disk separator, and pasteurized at 72 ˚C for 15 s. The pasteurized milk was subsequently preheated to 78 ˚C and evaporated in Niro three-effect falling film evaporator (GEA Niro A/S, Soeborg, Denmark) at sequential effect temperatures of 73 ˚C, 64 ˚C and 55 ˚C. Concentrate feed introduced to Niro Tall-Form Anhydro threestage spray dryer (GEA Niro A/S, Soeborg, Denmark) (air inlet temperature = 180 ˚C, air outlet temperature = 85 ˚C) at approximately 43% total solids (TS) with a centrifugal atomizer (GEA Niro A/S, Soeborg, Denmark) at Moorepark Technology Ltd, Fermoy, Co. Cork, Ireland. Primary and secondary fluidized beds were maintained at 74 ˚C and 24 ˚C, respectively. Fines were returned to the cyclone to the top of the spray dryer. Yielding low-heat non-agglomerated SMP of approximately 97% total solids, and a fat content <0.1%.

2.2.2 Consumer Acceptance Testing

2.2.2.1 Consumers

Consumers residing in Fuzhou (China, n = 106), Raleigh (USA, n = 100), and Cork (Ireland, n = 78) having similar characteristics in terms of gender, occupation (student) and age (18–30 years), participated in the consumer acceptance study (hedonic attribute testing). Consumers were regular self-reported consumers of milk, had experiences in drinking powdered milk products, and were non-rejecters of milk.

2.2.2.2 Evaluation Procedure

Milk powder samples were rehydrated at 10% solids (w/v) and stored at 4 ˚C overnight to allow powders hydrate. Preparations were conducted with the overhead lights off to prevent light-induced off-flavor formation. Consumers used the sensory hedonic attributes provided to them for the three different SMP samples (TMR, GRS, and CLV) presented three times. For consumer testing, samples were dispensed into 30 ml Styrofoam tumblers with three digit codes and taken from the refrigerator (4 ˚C) and served after 15 min at ambient temperature (20 ˚C). Each sample was served in a randomised balanced order with a 1 min rest between each sample. Consumers were asked to assess using a 9-point hedonic scale the liking of the following attributes: “appearance”, “aroma”, “overall”, “flavor”, “mouthfeel/thickness”, “creaminess” and “aftertaste” (hedonic). The consumers also rated the intensity of “freshness”, “cooked flavor”, aftertaste” and “quality” using a 5-point category scale anchored on the left with extremely low and on the right with extremely fresh (Stone, 2012). Sensory data was collected on paper ballots in Cork, Ireland and Fuzhou, China and by computerized data entry at Raleigh, USA.

2.2.3 Descriptive Analysis

2.2.3.1 Optimized Descriptive Profiling

Optimized Descriptive Profiling (ODP) (Silva et al. 2012, 2013; Silva et al. 2014) was only undertaken with panels in China and Ireland. ODP was chosen as a fully trained descriptive panel was not available in China. Chinese assessors were trained (O'Sullivan, 2016) in Fujian Agricultural and Forest University, China (n = 22) and Irish assessors were trained in University College Cork, Ireland (n = 25). These assessors were presented with all samples simultaneously but with randomised order to prevent first order and carry-over effects (MacFie et al. 1989). Assessors used the consensus list of sensory descriptors which were measured on a 10 cm line scale with the term "none" used as the anchor point for the 0 cm end of the scale and "extreme" for the 10 cm end of the scale (Table 2.1). For this study training and the use of a consensus sensory lexicon were used as described by Richter et al. (2010) and Faulkner et al. (2018) for a Ranking Descriptive Analysis technique. Sensory terms, which were the main sensory dimensions, were pre-selected from the sample set using an expert sensory panel (n = 10). Assessors evaluated the intensity of each attribute for each sample on the scales. Attributes were presented along with the table describing the sensory terms (Table 2.1). All samples were prepared in the same manner as the consumer analysis study and presented in triplicate.

Table 2.1 Sensory terms for optimised descriptive analysis of skim milk powder.

Descriptor	Explanation	Scale
Hedonic		
Appearance-Liking	The liking of appearance	0 = extremely dislike 10 = extremely like
Flavor-Liking	The liking of flavor	0 = extremely dislike 10 = extremely like
Aroma-Liking	The liking of aroma	0 = extremely dislike 10 = extremely like
Texture-Liking	The liking of texture	0 = extremely dislike 10 = extremely like
Overall acceptability	The acceptability of the product	0=extremely unacceptable 10 = extremely acceptable
Intensity		
Appearance-color	Appearance-Ivory to orange color	0 = Pale, 10 = Yellow
Sweet aroma	The smell associated with dairy sweet milky products	0 = none, 10 = extreme
Creamy aroma	The smell associated with creamy/milky products	0 = none, 10 = extreme
Cooked aroma	The smell associated with cooked milk products	0 = none, 10 = extreme
Oxidised aroma	The smell associated with rancid or oxidised products	0 = none, 10 = extreme
Painty aroma	The smell associated with rancid paint type notes	0 = none, 10 = extreme
Chalky Texture	Chalk like texture in the mouth	0 = none, 10 = extreme
Powdery Texture	Powdery texture in the mouth	0 = none, 10 = extreme
Viscosity	Thick texture in the mouth	0 = none, 10 = extreme
Sweet taste	Fundamental taste sensation of which sucrose is typical	0 = none, 10 = extreme
Cream flavour	The flavor associated with creamy/milky products	0 = none, 10 = extreme
Dairy sweet flavour	The flavors associated with sweetened cultured dairy products such as fruit yoghurt	0 = none, 10 = extreme
Dairy fat flavour	Intensity of fat flavor	0 = none, 10 = extreme
Oxidised flavour	The flavor associated with rancid or oxidised products	0 = none, 10 = extreme
Rancid butter	The flavor associated with rancid or oxidised butter	0 = none, 10 = extreme
Painty Flavor	The flavor associated with rancid paint type notes	0 = none, 10 = extreme
Fruity/Estery flavour	The flavors associated with fatty acid ethyl esters	0 = none, 10 = extreme
Cooked flavour	The flavor associated with cooked milk products	0 = none, 10 = extreme
Off-flavor	Off-flavor (Rancid)	0 = none, 10 = extreme
Astringent after-taste	Fundamental taste sensation of which aluminium sulphate is typical	0 = none, 10 = extreme

2.2.3.2. Descriptive Analysis

Descriptive sensory analysis (DA) was undertaken in the USA only. Rehydrated milk powders were evaluated in duplicate by seven trained panelists (Sensory Service Centre, North Carolina State University, USA), each with more than 120 h of experience in the descriptive analysis of dried dairy ingredients. Panelists evaluated the rehydrated milk powders using an established sensory lexicon (Table 2.2) and an intensity scale consistent with the Spectrum descriptive analysis method (Drake et al. 2003; Lloyd et al. 2009). All samples were prepared as per the consumer analysis study and presented in duplicate. In separate sessions, panelists evaluated coded samples in duplicate according to appropriate sensory practices. For analysis, each panelist evaluated the aroma of the sample and then tasted each sample. Samples were expectorated, and deionized water was used for palate cleansing.

Table 2.2 Sensory language for descriptive analysis of rehydrated skim milk powders.

Term	Definition	Reference	Example/preparation
Aroma intensity	The total orthonasal aroma impact		Evaluated as the lid is removed from the cupped sample
Sweet aromatic	Sweet aromatics associated with dairy products	Vanillin in milk	Vanilla cake mix or 20 ppm vanillin in milk
Cooked/milky	Aromatics associated with cooked milk	Cooked skim milk	Heating skim milk to 85 °C for 30 min
Cardboard/wet paper	Aromatics associated with wet cardboard or paper	Cardboard paper	Brown paper bag cut into strips and soaked in water
Grassy/hay	Aromatics associated with dried grasses	Alfalfa or grass hay	
Sweet taste	Fundamental taste sensation elicited by sugars	Sucrose	Sucrose (5% in water)
Salty taste	Fundamental taste sensation elicited by salts	Sodium chloride	Sodium chloride (0.5% in water)
Astringent	Fundamental taste sensation elicited by aluminium sulphate	Aluminum sulphate	Aluminum ammonium sulfate (0.09% in water)
Cowy/barny	Aromatics associated with cow feces and urin	p-cresol	20 ppm p-cresol in skim milk

2.2.2.4 Volatile Analysis

The SMP samples were analysed by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME GCMS) in triplicate. Four g of SMP sample (rehydrated at 10% solids (w/v)) was added to 20 ml amber screw capped headspace vials (Apex Scientific, Maynooth, Ireland) and equilibrated to 40 ˚C for 10 min with pulsed agitation of 5 sec at 500 rpm using a Shimadzu AOC 5000 Autosampler (Mason Technology Ltd, Dublin, Ireland). A single SPME fibre 50/30 μm Carboxen™/divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS), (Agilent Technologies Ltd, Cork, Ireland) was exposed to the headspace above the samples in the vial for 20 min at a depth of 1 cm at 40 ˚C. The fibre was retracted and injected into the gas chromatograph inlet and desorbed for 2 min at 250 ˚C. Injections were made on a Shimadzu 2010 Plus GC (Mason Technology Ltd, Ireland) with an DB-624 UI (60 m × 0.32 mm × 1.80 μm) (Agilent Technology Ltd, Ireland) column using a split/splitless injector in splitless mode with a merlin micro seal (Agilent Technology Ltd, Ireland). The temperature of the column oven was set at 40 ˚C, held for 5 min, increased at 5 ◦C min^{-1} to 230 ˚C then increased at 15 ˚C min^{-1} to 260 ˚C, yielding at total run time of 65 min. The carrier gas was helium held at a constant flow of 1.2 ml min^{-1}. The detector was a Shimadzu TQ8030 mass spectrometer detector (Mason Technology Ltd, Ireland), ran in single quad mode. The ion source temperature was 220 ˚C and the interface temperature was set at 260 ˚C. The mass spectrometer mode was electronic ionization (70v) with the mass range scanned between 35 and 250 amu. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, a commercial flavor and fragrance library (FFNSC Shimadzu, Mason Technology Ltd, Ireland) and an in-house library created in Shimadzu GCMS Solutions software (Mason Technology Ltd, Ireland) with target and qualifier ions and

linear retention indices for each compound. Linear retention indices were performed as per the method of Van den Dool and Kratz (1963). Spectral deconvolution was also performed to confirm the identification of compounds using AMDIS software (v 2.3, Gaithersburg, MD, USA). Ten ul of a standard solution (1-butanol, dimethyl disulphide, butyl acetate, cyclohexane, benzaldehyde) at 10 ppm, and 2-phenylethanol at 50 ppm in methanol: water (1:99) were run before and after every series of samples to ensure that both the SPME extraction and MS detection were performing within specification. An auto-tune of the GCMS was carried out prior to the analysis to ensure optimal performance. Results were expressed as abundance values only which represent peak areas, due to the difficulty of quantification using HS-SPME where multiple chemical classes are present in the sample.

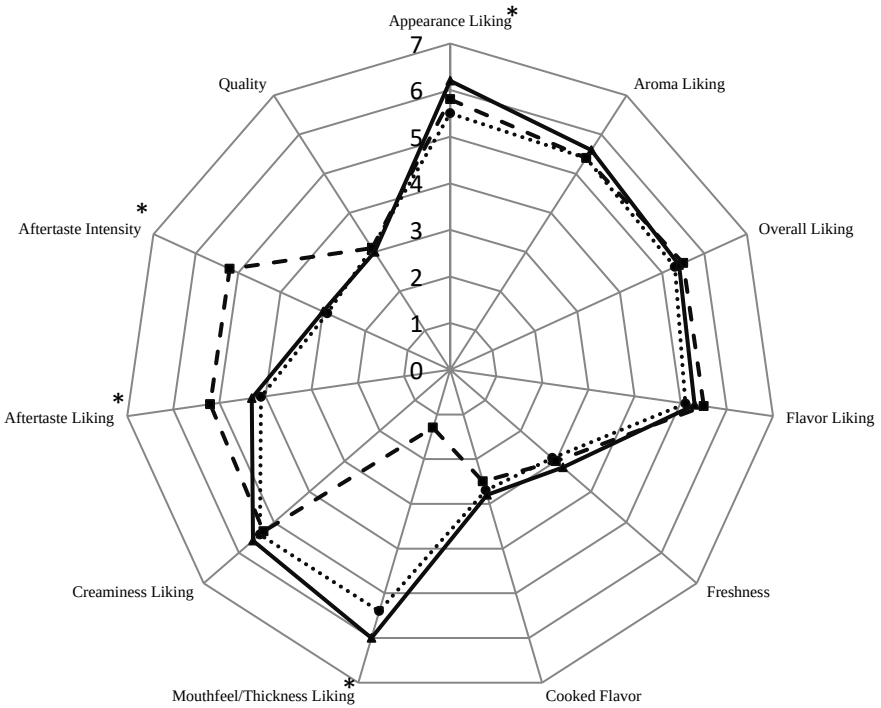
2.2.2.5 Statistical Analysis

The statistical analysis of the volatile compounds was undertaken using Kruskal-Wallis-Partial Least Squares Regression (KW-PLSR). PSLR combines features of Principal Component Analysis (PCA) and multiple regressions and is performed separately on a set of dependent variables from a large set of independent variables. Based on the PSLR Beta coefficients results, the compound which has a significantly higher ($p < 0.05$) absolute value of the beta coefficient (Arjo, 2009) was used in the Kruskal-Wallis H test (one-way ANOVA on ranks) to evaluate the significant differences among the SMP samples for each volatile compound. To classify SMP samples in a supervised multivariate model, partial least squares discriminant analysis (PLS-DA) was performed. The Variable Importance Plot (VIP) scores summarised the individual X variables and have an influence on the PLS model and rank in terms of importance on the Y axis (with variables of the highest importance at the top) (Strobl et al. 2007). VIP scores give a measure useful to show the volatile

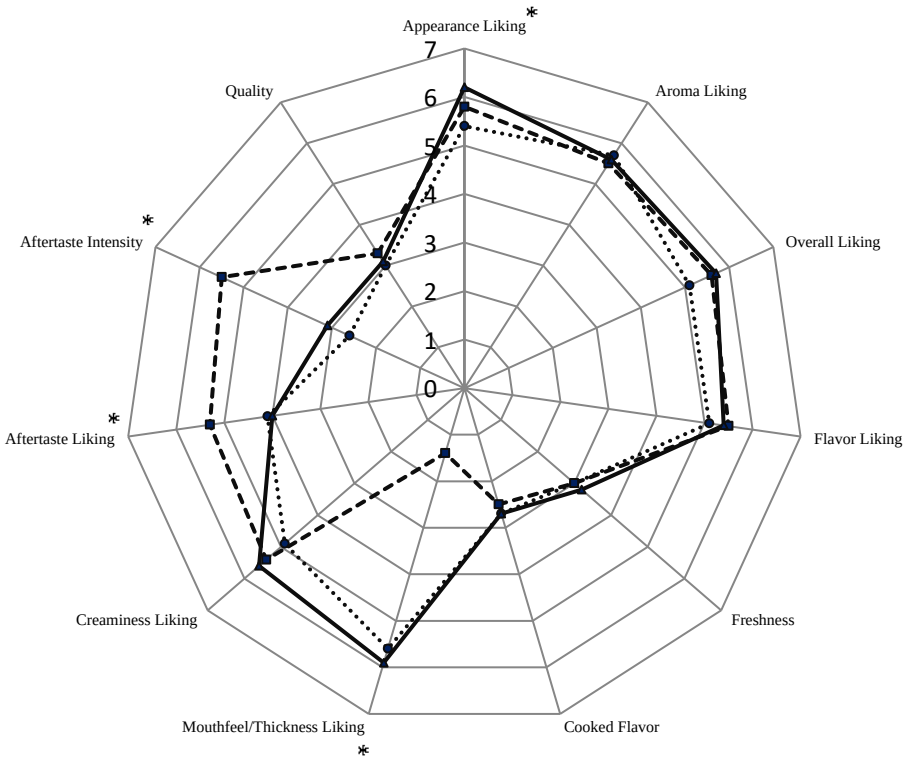
compounds' latent variables that contribute most to the underlying variation in feeding systems. Unsupervised hierarchical clustering analysis was used to show the patterns in the volatile data and is presented as a heatmap. A heatmap can be created by assigning a color to the individual values contained in a matrix (Liu et al. 2012) and provided an intuitive visualisation of a data table. Consumer acceptance data obtained from the sensory trials were evaluated by oneway ANOVA using cultural differences of perception and feeding systems as primary factors. The post hoc Tukey test was performed to find which feeding systems were significantly different among different cross cultural consumers (Ireland, USA and China). The SPSS V23.0 (IBM Statistics Inc., Armonk NY) was used for Kruskal-Wallis H test and oneway ANOVA. Descriptive data was evaluated by analysis of variance with means separation (SAS version 9.2, Cary, North Carolina, USA). Unscrambler X software, version 10.3 (CAMO ASA, Trondheim, Norway) was used for ANOVA-PSLR (APLSR) analysis of milk powders data in different feeding systems and variance of ODP sensory data to process the raw data accumulated from the +20 test subjects (Figure. 2.1). The X-matrix was designed as 0/1 design variables for treatment*nationality. The Y-matrix was designed as sensory variables. The optimal number of components in the APLSR models presented was determined to be 4 Principal Components (Figure. 2.2). PC 1 versus PC 2 is presented; the other PC's did not yield additional information or provide any predictive improvement in the Y-matrix obtained through their examination. To derive significance indicators for the relationships determined in the quantitative APLSR, regression coefficients were analyzed by jackknifing (Table 2.3) which is based on cross-validation and stability plots (Martens & Martens, 1999, 2000, 2001a, 2001b). All analyses were performed using the Unscrambler Software, version 10.3 (CAMO ASA, Trondheim, Norway). MetaboAnalyst 4.0 (McGill University,

Montreal, QC, Canada) software was used to perform the Variable importance plot (VIP), PLS-DA and Hierarchical clustering analysis (HCA) (Chong et al. 2018).

(a)


Appearance Liking*
Aroma Liking
Overall Liking
Flavor Liking
Freshness
Cooked Flavor
Mouthfeel/Thickness Liking*
Creaminess Liking
Aftertaste Liking*
Aftertaste Intensity*
Quality
0
1
2
3
4
5
6
7

(b)


Appearance Liking
*
Aroma Liking
Overall Liking
Flavor Liking
Freshness
Cooked Flavor
Mouthfeel/Thickness Liking
*
Creaminess Liking
Aftertaste Liking
*
Aftertaste Intensity
*
Quality
7
6
5
4
3
2
1
0

(c)

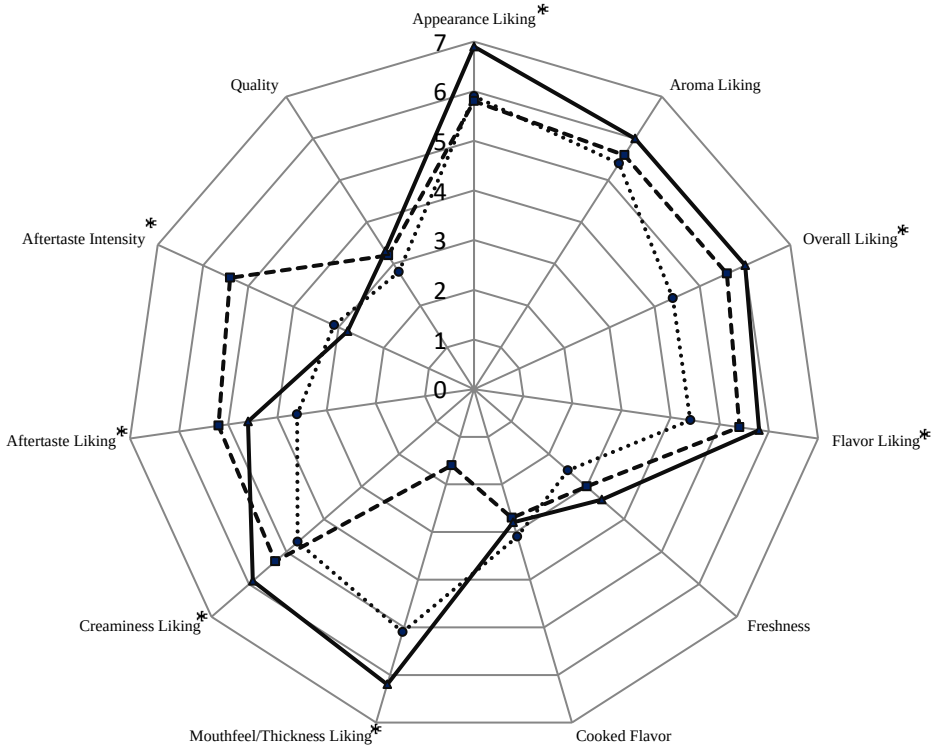


Figure 2.1 Sensory hedonic analysis performed by Irish (●) ,Chinese (■) and USA (▲) consumers' on reconstituted skim milk power derived from three distinct diets; (a) clover (CLV), (b), grass (GRS), and (c) total mixed ration (TMR). Significantly different attributes are marked with an asterisk ($p<0.05$).

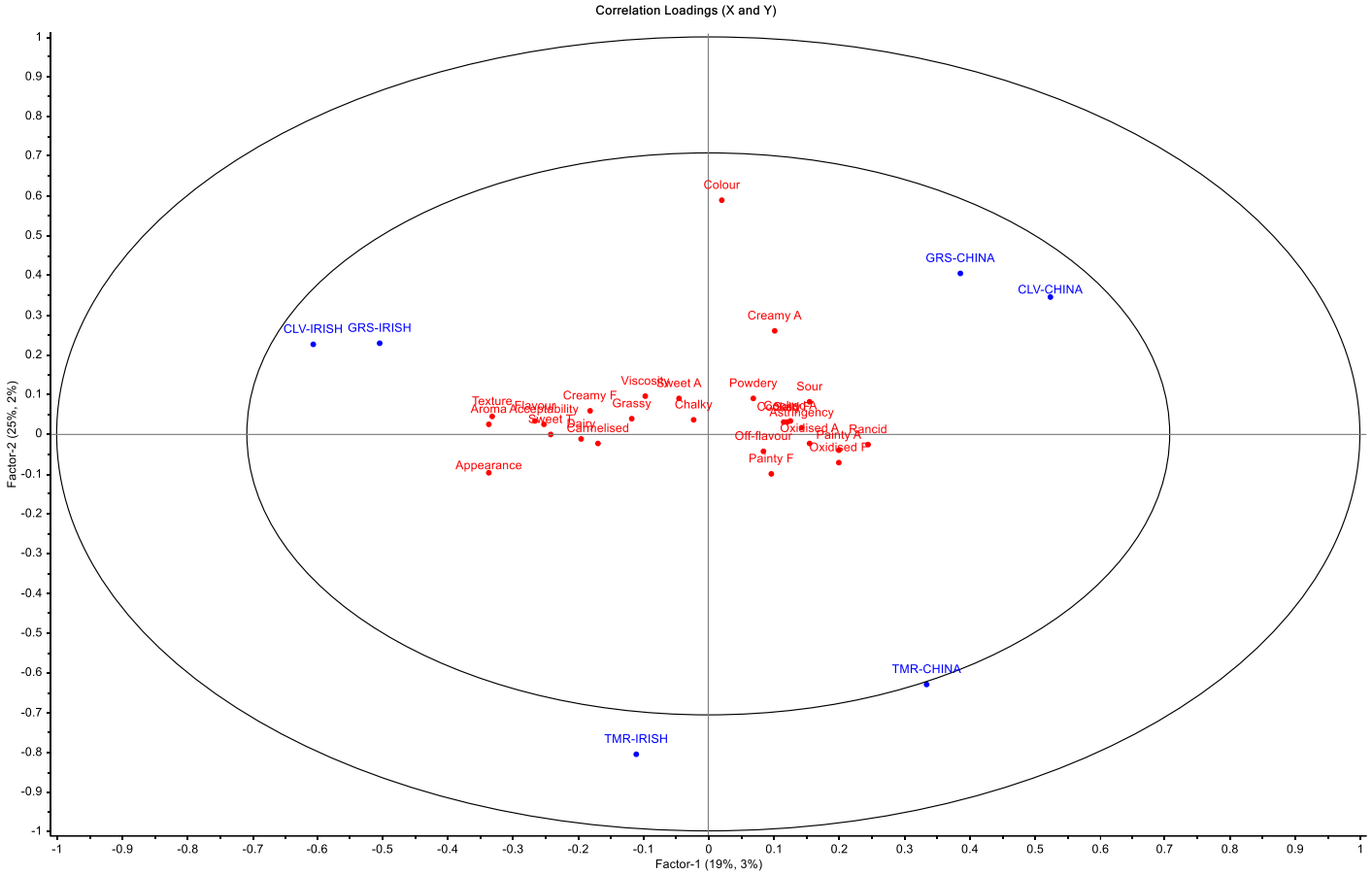


Figure 2.2 Optimized Descriptive Profiling (ODP). ANOVA-Partial Least Squares regression (APLSR) for skim milk powder produced outside on grass (GRS), grass/clover (CLV) or indoors on trial mixed rations (TMR) by Chinese and Irish panelists. The X-matrix was designed as different cultural consumers' perceptions. The Y – matrix involved the sensory, variables of the design.

Table 2.3 P-values of regression coefficients from ANOVA-Partial Least Squares regression (APLSR) for hedonic and intensity sensory terms of skim milk powders produced from different diets (CLV, GRS and TMR) by Chinese and Irish consumers on Optimized Descriptive Profiling (ODP) .

Attributes	Sample					
	CLV-CHINA	GRS-CHINA	TMR-CHINA	CLV-IRISH	GRS-IRISH	TMR-IRISH
Appearance	-0.98784***	-0.79825**	-0.40235^{NS}	0.836526***	0.662394^{NS}	0.536913**
Aroma	-0.76125***	-0.55272*	-0.62958*	0.85696***	0.69973***	0.152608^{NS}
Flavor	-0.61571*	-0.4379^{NS}	-0.55604*	0.724232***	0.593686***	0.085165^{NS}
Texture	-0.74959**	-0.53176^{NS}	-0.68392**	0.886347***	0.726911***	0.097986^{NS}
Acceptability	-0.5836*	-0.42079^{NS}	-0.49772*	0.666983***	0.545359***	0.10469^{NS}
Color	0.876854**	1.058541*	-1.43414^{NS}	0.447739^{NS}	0.473134*	-1.94062^{NS}
Sweet Aroma	-0.002^{NS}	0.06389^{NS}	-0.33607^{NS}	0.224464^{NS}	0.199937^{NS}	-0.27293^{NS}
Creamy Aroma	0.65794**	0.672829^{NS}	-0.45454**	-0.07705^{NS}	-0.01318^{NS}	-0.94813**
Cooked Aroma	0.455909*	0.365415^{NS}	0.201013^{NS}	-0.39625^{NS}	-0.31478^{NS}	-0.23528^{NS}
Oxidised Aroma	0.386657^{NS}	0.271352^{NS}	0.36782^{NS}	-0.46719*	-0.38387^{NS}	-0.03825^{NS}
Painty Aroma	0.420726**	0.289852^{NS}	0.42792*	-0.52676**	-0.43409**	-0.01899^{NS}
Chalky	-0.0128^{NS}	0.015315^{NS}	-0.13655^{NS}	0.09811^{NS}	0.086382^{NS}	-0.10038^{NS}
Powdery	0.28357^{NS}	0.265564^{NS}	-0.0709^{NS}	-0.11628^{NS}	-0.07973^{NS}	-0.30647^{NS}
Viscosity	-0.1256^{NS}	-0.03534^{NS}	-0.38976^{NS}	0.331355^{NS}	0.284798^{NS}	-0.20848^{NS}
Sweet Taste	-0.71052*	-0.53002**	-0.51528^{NS}	0.751774**	0.61024**	0.201569^{NS}
Sour	0.484203**	0.409785^{NS}	0.102463^{NS}	-0.34707^{NS}	-0.26855*	-0.34062^{NS}
Salty	0.383679^{NS}	0.306636^{NS}	0.173699^{NS}	-0.33649^{NS}	-0.2676^{NS}	-0.1943^{NS}
Creamy Flavor	-0.41251^{NS}	-0.26668^{NS}	-0.50917*	0.576011*	0.478696*	-0.05463^{NS}
Dairy	-0.55868*	-0.42517*	-0.36211^{NS}	0.562512**	0.454328**	0.193685^{NS}
Caramelized	-0.55975*	-0.4354*	-0.3146^{NS}	0.531564*	0.426648*	0.233449^{NS}
Oxidised Flavor	0.448648^{NS}	0.285196^{NS}	0.578607**	-0.64297**	-0.53534**	0.079697^{NS}
Rancid	0.640691**	0.458645^{NS}	0.563349*	-0.74348**	-0.60874**	-0.10108^{NS}
Painty Flavor	0.110838^{NS}	0.022811^{NS}	0.386808^{NS}	-0.32089^{NS}	-0.27671^{NS}	0.219001^{NS}
Grassy	-0.22716^{NS}	-0.14647^{NS}	-0.28238^{NS}	0.318521*	0.264788^{NS}	-0.03171^{NS}
Cooked Flavor	0.422567^{NS}	0.338218^{NS}	0.188731^{NS}	-0.36888^{NS}	-0.29319^{NS}	-0.21609^{NS}
Off-Flavor	0.157343^{NS}	0.091127^{NS}	0.248433^{NS}	-0.25574^{NS}	-0.21471^{NS}	0.065148^{NS}
Astringency	0.389324^{NS}	0.299208^{NS}	0.237368^{NS}	-0.38205^{NS}	-0.30774*	-0.14721^{NS}

Rows differing in asterisk are statistically different, where the significance of regression coefficients *=p<0.05,**=p<0.01,***=p<0.001. NS= not significant

CLV = grass/clover, GRS = grass, and TMR = total mixed ration.

2.3 Results and Discussion

2.3.1 Sensory Evaluation

2.3.1.1 Irish, USA and Chinese Consumer Acceptance Testing

The results of the consumer acceptance study of SMP based on different forage types are presented in Figure.2.1a,b,c. Significant cross-cultural differences were evident in the perception of the SMP based on diet between the different cultural groups. Chinese consumers rated all SMP dietary types (Figure.2.1a,b,c) statistically ($p < 0.05$) higher for "aftertaste liking" and "aftertaste intensity" and lower for "mouthfeel/ thickness liking". In contrast, USA consumers scored all SMP dietary types significantly higher ($p < 0.05$) for "appearance liking". Moreover, USA consumers also scored "flavor liking", "creaminess liking", "overall liking" and "mouthfeel/thickness liking" statistically higher ($p < 0.05$) in the TMR SMP sample (Figure.2.1 c), while Irish consumers scored these same attributes significantly lower ($p < 0.05$) in the TMR SMP sample.

The mean scores for hedonic attributes with different diets of SMP for Irish, USA and Chinese consumers are also shown in Table 2.4. The USA consumers preferred the TMR SMP and found no significant difference ($p < 0.05$) between CLV and GRS SMP, apart from a higher preference ($p < 0.05$) for GRS SMP for "overall liking" and "flavor liking". Irish and USA consumers had similar perceptions of GRS SMP and CLV SMP apart from "appearance liking", "creaminess liking" and "mouthfeel/thickness liking", with USA consumers also scoring the GRS SMP higher ($p < 0.05$) for "overall liking", "flavor liking" and "aftertaste intensity" than the CLV SMP. Irish consumers generally gave higher scores for SMP derived from pasture fed milk ($p < 0.05$) for most attributes, except for "appearance-liking", "cooked flavor

intensity" and "aftertaste intensity". No significant differences ($p < 0.05$) were observed between the SMP based on the diet by Chinese consumers, apart from a higher score for "cooked flavor intensity" for the TMR SMP ($p < 0.05$).

Differences in perception between the cultural consumer groups are likely related to product familiarity, as it has been shown to be the significant factor delineating consumer preference tendencies in a crosscultural context (de Albuquerque et al. 2018; Torrico et al. 2019). It is also thought that the cross-cultural difference in the frame of reference is especially influenced by previous exposure (Kim et al.2015; Kim et al. 2018). It is possible that the heat-treatment used in SMP production may also be impacting on sensory perception across the cultural groups. Previous studies have shown that USA and Chinese consumers are more familiar with higher heat-treated dairy products (Song and Kaiser, 2016) and other studies have reported that the nature of the heat treatment used in pasteurization of dairy products has an impact on specific sensory attributes; such as "cooked flavor", "quality", "aftertaste" and "astringency" (Lee et al. 2017; Schiano et al. 2017). As most retail liquid milk consumption in China is ultra-high temperature (UHT) to overcome transport challenges, extend shelf life and reduce costs (Liem et al. 2016), Chinese consumers are likely more familiar with high heat-treated dairy products. Our data shows that USA consumers gave higher scores for "appearance liking", "creaminess liking" and "mouthfeel/thickness liking" in comparison to Irish and Chinese consumers, thus indicating that the extent of temperature of heat-treatment was not negatively impacting on these sensory attributes for USA consumers. However, Irish consumers who are more used to high temperature short time (HTST) pasteurization (72 ◦C, 15 s) of dairy products (Anon, 2018), may not be as familiar with a high heat-treated (180 ◦C) SMP type product and this may account for their general lower scores

for many of the sensory attributes in comparison to Chinese and USA consumers (Table 2.4).

USA consumers also scored "appearance liking" significantly higher ($p < 0.05$) for all SMP irrelevant of diet, with the highest score for SMP from TMR. It appears that USA consumers preferred whiter color dairy powders and this may also be due to familiarity as most of the dairy products in the USA are produced from cows fed indoors on TMR rather than pasture. Valverde Pellicer (2007) reported that color is a suitable biomarker to distinguish the appearance of TMR and pasture-fed milk. In previous studies, the yellow color was statistically higher ($P < 0.05$) in milk and butter from pasture-fed cows than from a TMR diet, and directly correlated with tran-β-carotene content (O'Callaghan et al. 2016a; Faulkner et al. 2018). USA consumers also preferred the TMR SMP ($p < 0.05$) for "aroma liking", "overall liking", "flavour liking", "freshness intensity", "creaminess liking" and "aftertaste liking" also indicating that preference is based on familiarity.

Chinese consumers scored higher for "aftertaste liking" and "aftertaste intensity" and lower for "mouthfeel/thickness liking" than the USA and Irish consumers for SMP from all diets. Zhi et al. (2016) found that a high "aftertaste intensity of thickness and sweetness" is often used as a positive term to describe better quality milk in China and thus the concept of 'aftertaste' may be cultural dependent, because the underlying conceptual elements and words used to describe its features may be dissimilar. Clark et al. (2009) reported that good quality milk should have a pleasantly sweet and clean flavor with no distinct aftertaste, and Porubcan and Vickers (2005) found that the aftertaste of milk significantly contributes to the dislike of milk among Western consumers that avoid drinking milk. As the attribute "aftertaste liking" was influenced by diet for Chinese consumers the result may suggest that differences

may have more to do with the verbalisation of sensory perception and linguistic representation, rather than due to the Western definition of the term. Difficulties associated with transmitting sensory concepts among countries have been previously described with some words difficult to categorise with ambiguous cultural meanings (Son et al. 2014).

Table 2.4 The mean scores of consumer acceptance analysis of skim milk powders from Irish, Chinese and USA consumers.

Sensory attributes	I-CLV	I-GRS	I-TMR	C-CLV	C-GRS	C-TMR	U-CLV	U-GRS	U-TMR
Appearance Liking	5.5^{d}	5.4^{d}	5.9^{c}	5.8^{c}	5.8^{c}	5.8^{c}	6.2^{b}	6.2^{b}	6.9^{a}
Aroma Liking	5.4^{b}	5.7^{b}	5.4^{b}	5.4^{b}	5.5^{b}	5.6^{b}	5.6^{b}	5.6^{b}	6^{a}
Overall Liking	5.3^{c}	5.1^{d}	4.4^{e}	5.5^{b}	5.6^{b}	5.6^{b}	5.4^{c}	5.7^{b}	6^{a}
Flavor Liking	5.1^{c}	5.1^{c}	4.4^{d}	5.5^{b}	5.5^{b}	5.4^{b}	5.3^{c}	5.4^{b}	5.8^{a}
Freshness intensity	2.9^{b}	3^{b}	2.5^{c}	3^{b}	3^{b}	3^{b}	3.2^{b}	3.2^{b}	3.4^{a}
Cooked Flavor	2.7^{b}	2.7^{b}	3.1^{a}	2.5^{c}	2.5^{c}	2.7^{b}	2.8^{b}	2.7^{b}	2.8^{b}
Mouthfeel/Thickness Liking	5.4^{c}	5.6^{c}	5.1^{c}	1.3^{d}	1.4^{d}	1.6^{d}	6^{a}	5.9^{a}	6.2^{a}
Creaminess Liking	5.4^{c}	4.9^{d}	4.7^{d}	5.3^{c}	5.4^{c}	5.3^{c}	5.6^{b}	5.6^{b}	5.9^{a}
Aftertaste Liking	4.1^{c}	4.1^{c}	3.6^{d}	5.2^{a}	5.3^{a}	5.2^{a}	4.3^{c}	4^{c}	4.6^{b}
Aftertaste Intensity	2.9^{b}	2.6^{c}	3.1^{b}	5.2^{a}	5.5^{a}	5.4^{a}	3^{b}	3.1^{b}	2.8^{b}
Quality	3.1^{a}	3^{a}	2.8^{a}	3.1^{a}	3.3^{a}	3.2^{a}	3^{a}	3.1^{a}	3.3^{a}

Means within a row not sharing a superscript letter are significantly different ($p<0.05$, post hoc Tukey test)

I denote Irish, C denotes Chinese, and U denotes USA consumers.

CLV = grass/clover, GRS = grass, and TMR = total mixed ration.

Liking attributes were scored on a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely.

Intensity and quality were scored on a 5 point scale where 1 = extremely low and 5 = extremely high.

2.3.1.2 Optimized Descriptive Profiling – Irish and Chinese Trained Assessors

The ODP evaluation of SMP from different diets is shown in the APLSR plot, (Figure.2.2). The SMP samples were subdivided into three distinct cluster groups based on the evaluations by the Irish and Chinese assessors. No significant sensory differences ($p < 0.05$) were evident between SMP derived from GRS and CLV diets for both Chinese and Irish assessors, while both groups discerned that TMR SMP was significantly ($p \leq 0.05$) different to SMP from GRS and CLV diets.

The significance (p-value) of regression coefficients for the ODP attributes described by the Irish and Chinese assessors for SMP from different diets (CLV, GRS and TMR) are illustrated in Table 2.3. The CLV SMP and GRS SMP samples were rated significantly higher for "creamy flavor" ($p < 0.05$), "dairy sweet flavor" ($p < 0.01$), "sweet taste" ($p < 0.01$) and "caramelized flavor" ($p < 0.05$) by Irish assessors, which again may be linked to familiarity with pasture derived dairy products. It is interesting that the Irish assessors significantly scored "sweet taste" ($p < 0.05$) higher for both CLV and GRS SMP and that Chinese assessors found a negative perception of "sweet taste" for both CLV ($p < 0.05$) and GRS ($p < 0.01$) SMP, as previously Chung et al. (2010) determined that the optimal sweetness level for a sensory evaluated sports-drink was lower for American than Asian consumers, perhaps displaying a higher propensity for sweet beverages in the latter group.

The Chinese assessors scored the color ("appearance-color) of GRS ($p < 0.05$) and CLV SMP ($p < 0.01$) significantly higher than TMR SMP, while the Irish assessors only rated a significantly higher score for the color of GRS SMP ($p < 0.05$) (but did give a numerically greater score for CLV than TMR SMP) (Table 2.3). Chinese assessors scored "creamy aroma" ($p < 0.01$), "sour taste" ($p < 0.01$) and

"cooked aroma" significantly ($p<0.05$) higher for the CLV SMP sample, and both Irish and Chinese assessors had a significantly ($p < 0.01$) negative perception of "creamy aroma" in TMR SMP. Irish assessors had a significant negative score for "sour taste" ($p < 0.05$) for GRS SMP. "Oxidised aroma" was statistically negatively ($p < 0.05$) associated with the CLV SMP sample by Irish assessors, who also found that "oxidised flavor" and "rancid butter" were negatively associated with the CLV ($p < 0.01$) and GRS ($p < 0.01$) SMP. Irish assessors also found a negative association of "painty aroma" with both CLV and GRS ($p < 0.01$) SMP, indicating further preferences for CLV and GRS over TMR SMP. Chinese assessors scored "painty aroma" and "rancid butter" higher in CLV ($p < 0.01$) and TMR ($p < 0.05$) and had a significantly higher score ($p < 0.01$) for "oxidised flavour" in the TMR SMP than Irish assessors. "Astringent after-taste" in GRS SMP was significantly ($p < 0.05$) negatively correlated by Irish assessors and who also significantly ($p < 0.05$) correlated "grassy flavor" to only the CLV SMP. This is in partial agreement with Croissant et al. (2007) who found greater intensities of grassy and cowy/barny flavors in pasture based milks compared with TMR milks when evaluated at 15 °C using trained panelists. Similarly, the present results are in partial agreement with Villeneuve et al. (2013), who using a sensory ranking test, found that percentage of assessors ranking for the intensity of grassy (grass, leafy vegetable, and plant) flavors was higher for milk from cows fed pasture compared with hay and silage. The other sensory attributes ("sweet aroma", "chalky texture", "powdery texture", "viscosity", "salty taste", "painty flavor", "cooked flavor" and "off-flavor") were not significantly different between Chinese and Irish assessors.

2.3.1.3 Descriptive Analysis by USA Trained Panelists

The CLV and GRS SMP samples scored statistically higher ($P < 0.05$) for "grassy/hay" (highest in CLV SMP samples) and the GRS SMP sample scored statistically highest ($P < 0.05$) for "salty taste" (Figure.2.3). In contrast, the TMR SMP scored significantly higher ($p < 0.05$) for "sweet aromatic". "Cardboard/wet paper" was only detected in the CLV SMP. The panel also commented on the fact that the CLV SMP and GRS SMP samples had a pronounced "cowy/barny" attribute which was absent in the TMR SMP sample. As stated previously a "barnyard" aroma in milk was associated with pasture diets, and found to be highest in CLV milk (Faulkner et al. 2018). Grassy and hay flavors have been previously documented by trained US panelists in dairy products from cows fed pasture based diets (Croissant et al. 2007; Drake et al. 2005; Drake et al. 2009). The USA panelists did not find any signficant differences in "aroma intensity", "cooked/milky", "sweet taste" or "astringent" between the CLV, GRS and TMR SMP.

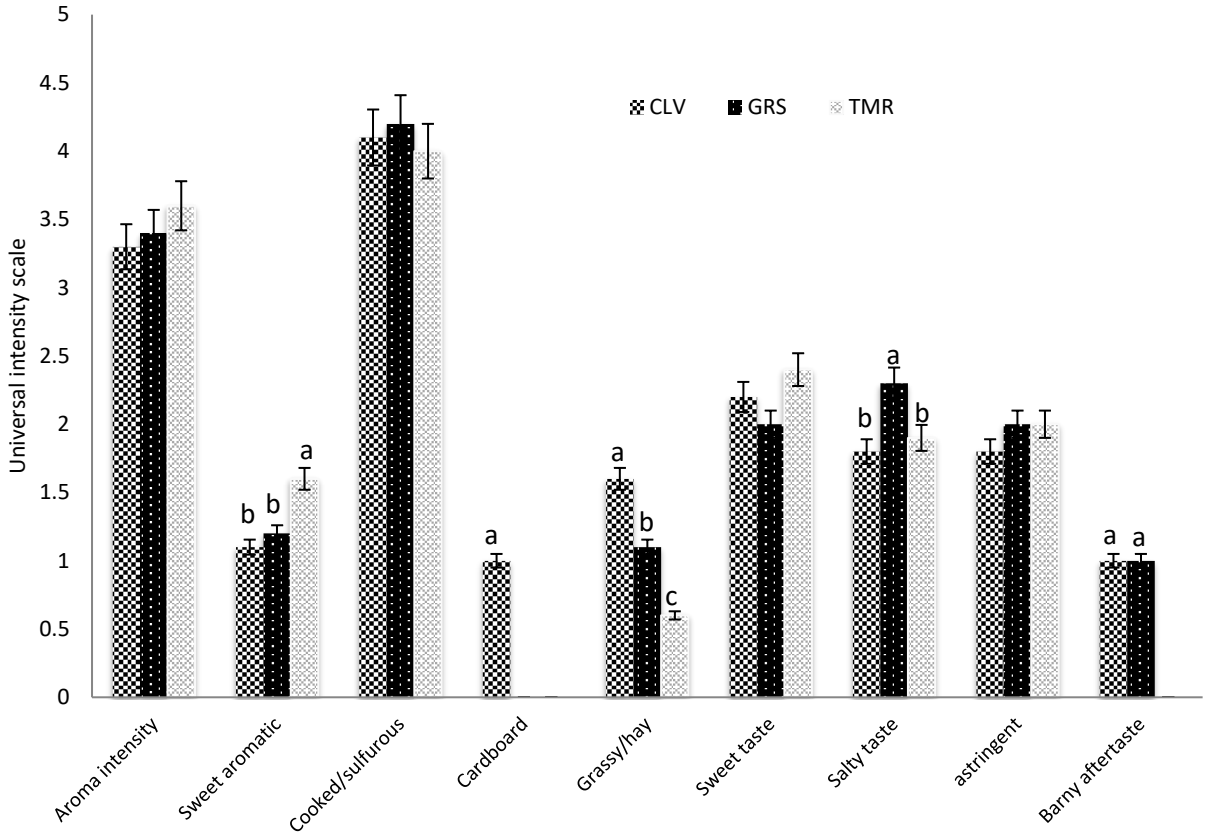


Figure 2.3 Descriptive sensory trained attribute intensities of rehydrated SMP derived from different diets (GRS, CLV & TMR) by USA assessors (n=10). The error bars represent mean standard error within replicates. Columns with different letters (a-c) for each attribute are statistically different ($p<0.05$). Intensities were scored on a 0 to 15-point universal scale, most dairy powder flavors fall between 0 and 4 on this scale (Drake et al. 2009).
CLV = grass/clover, GRS = grass, and TMR = total mixed ration.

2.3.2 Volatile Analysis

Diet was shown to have a significant effect ($p < 0.005$) on the volatile compounds of SMP (Figure. 2.4). In total, 26 volatile compounds were identified in these SMP samples (Table 2.5). The TMR SMP samples had the greatest abundance of acetic acid, nonanal, decanal and acetoin ($p < 0.01$), and 1-hydroxy-2-propanone (hydroxyacetone) and propanoic acid ($p < 0.05$). The CLV SMP had the greatest abundance of ethanol, dimethyl sulfone ($p < 0.05$), pentanal and heptanal ($p < 0.01$). No significant differences ($p < 0.05$) were observed between diets for butanoic acid, 1-butanol, 1-pentanol, phenol, 2-ethyl-1-hexanol, benzyl alcohol, hexanal, benzaldehyde, 2-pentylfuran, acetone, 2-butanone, ε-caprolactone, 2-undecanone, α-pinene, 3-carene and toluene. Variable importance coefficients (VIP) scores highlight those volatiles contributing most to the observed discrimination (Figure.2.5). Volatiles with a VIP ≥ 0.80 discriminating TMR SMP were predominantly metabolic carbohydrate products such as acetoin and acetic acid, which were highest in TMR SMP and lowest in CLV SMP. Moreover, the GRS and CLV SMP samples had greater levels of dimethyl sulfone, heptanal, pentanal and 2-undecanone arising from protein metabolism and lipid oxidation, respectively.

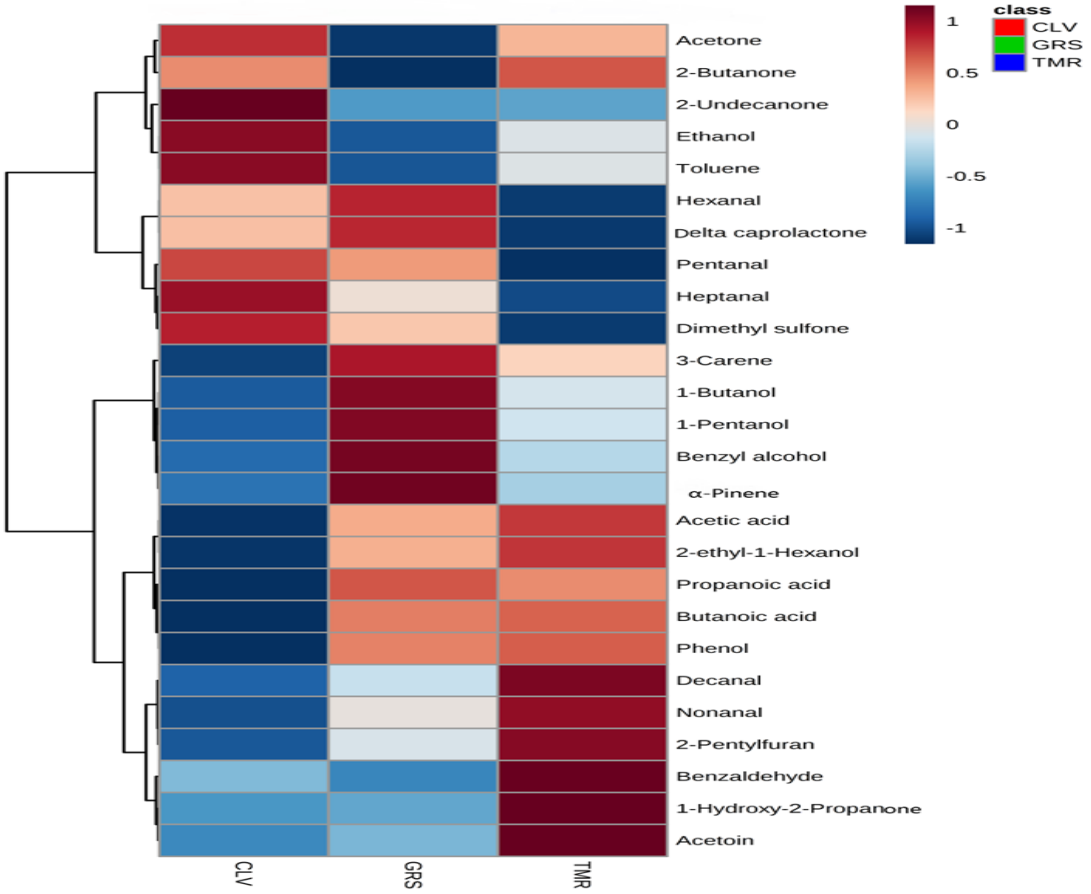


Figure 2.4 Hierarchical clustering analysis (heatmap) of volatile compounds derived from different feeding systems; grass (GRS) , grass/clover (CLV) and total mixed ration (TMR). Statistics by ANOVA with post hoc Turkey test. The degree of positive and negative correlation of SMP is indicated by +1 (red) to -1 (blue).

Table 2.5 Volatile compounds identified by HS-SPME-GCMS in SMP produced from different diets [grass (GRS), grass/clover (CLV) and total mixed rations (TMR)]; values indicate abundance values for each compound.

Volatile compound	CAS no.[1]	LRI [1]	CLV	GRS	TMR	P-value
Acid						
Acetic acid	64-19-7	687	4.47E+04	7.55E+04	9.07E+04	***
Propanoic acid	79-09-4	777	9.35E+03	1.59E+04	1.60E+04	*
Butanoic acid	107-92-6	859	1.16E+05	1.60E+05	1.73E+05	NS
Alcohol						
Ethanol	64-17-5	512	9.12E+04	7.68E+04	8.27E+04	*
1-Butanol	71-36-3	713	3.78E+04	3.93E+04	3.95E+04	NS
1-Pentanol	71-41-0	813	4.47E+03	6.65E+03	5.07E+03	NS
Phenol	108-95-2	1092	1.03E+04	1.16E+04	1.17E+04	NS
2-Ethyl-1-hexanol	104-76-7	1073	3.96E+05	4.32E+05	4.43E+05	NS
Benzyl alcohol	100-51-6	1119	1.17E+04	1.22E+04	1.20E+04	NS
Aldehyde						
Pentanal	110-62-3	734	1.10E+04	9.77E+03	6.51E+03	***
Hexanal	66-25-1	837	4.38E+04	4.39E+04	4.21E+04	NS
Heptanal	111-71-7	941	2.06E+04	1.63E+04	1.25E+04	***
Benzaldehyde	100-52-7	1027	2.01E+04	1.88E+04	2.65E+04	NS
Nonanal	124-19-6	1146	5.98E+04	7.04E+04	8.24E+04	***
Decanal	112-31-2	1250	8.12E+03	9.08E+03	1.09E+04	***
Furan						
2-Pentylfuran	3777-69-3	1043	1.89E+03	2.51E+03	3.52E+03	NS
Ketone						
Acetone	67-64-1	535	1.64E+05	1.17E+05	2.00E+05	NS
2-Butanone	78-93-3	636	1.94E+04	1.56E+04	2.43E+04	NS
1-Hydroxy-2-Propanone	116-09-6	733	2.17E+04	2.17E+04	4.07E+04	*
Acetoin	513-86-0	776	1.84E+03	3.54E+03	9.00E+04	***
lactone	502-44-3	1276	2.09E+04	2.21E+04	1.92E+04	NS
2-Undecanone	112-12-9	1344	7.26E+03	2.83E+03	3.13E+03	NS
Sulfur compound						
Dimethyl sulfone	67-71-0	1051	2.07E+05	1.81E+05	1.23E+05	*
Terpene						
α-Pinene	80-56-8	952	2.77E+04	3.32E+04	2.99E+04	NS
3-Carene	13466-78-9	1031	2.69E+04	3.14E+04	2.93E+04	NS
Phenyl						
Toluene	108-88-3	794	4.50E+03	4.51E+03	6.73E+03	NS

[1]CAS no.= Chemical Abstracts Service number. LRI = linear retention index

Kruskal-Wallis Ranking test statistical analysis:* and *** denote significant differences at $P<0.05$ and $P<0.001$, respectively.

Short chain carboxylic acids (SCFFA) are derived from carbohydrate metabolism, lipolysis or amino acid metabolism but may also be transferred directly from forage due to their volatility (Kilcawley et al., 2018) and contribute to the sour and cheese flavor of milk (Parker, Elmore, & Methven, 2014). Acetic and propanoic acid were statistically higher in SMP from the TMR diet in comparison to SMP from GRS and CLV (Table 2.5). This is most likely due to the higher carbohydrate content of TMR which is metabolised in the rumen to these short-chain FFA (Coppa et al. 2011). The CLV SMP samples were perceived as having the highest score for both "cardboard/wet paper" and "cowy/barny" aftertaste by DA analysis by USA panelists (Figure. 3). Pentanal and heptanal are primary products of lipid oxidation and associated with "cardboard" flavor (Whitson, Miracle, & Drake, 2010) and were significantly higher in the CLV SMP than the SMP derived from either GRS or TMR. Park and Drake (2016) found that "cardboard" flavor was inversely proportional to a lower "sweet aromatic" flavor in SMP, it worth nothing that "cardboard/ wet paper flavor" as detected by USA panelists in the CLV SMP also had the lowest intensity of "sweet aromatic" aroma (Figure 2.3). As stated, nonanal and decanal are also products of lipid-oxidation and were statistically higher ($P < 0.05$) in SMP derived from TMR, Chinese consumers also found that "oxidised flavor" was statistically higher for SMP TMR. Boltar et al. (2015) found nonanal and decanal were significantly higher in winter Nanos cheese and suggested that these compounds are not present in pasture-fed products. Park et al. (2013) found that nonanal and decanal had been attributed to "off-flavor (cardboard, fatty)" during UHT processing.

In ruminants, ethanol is oxidized to acetaldehyde by alcohol dehydrogenase and has been shown to be directly transferred from forage (maize silage) to milk (Randby et al.1999). Toso et al. (2002) also demonstrated that ethanol had a significant

role in milk derived from preserved forages and was a major discriminator. Our study indicated that ethanol was in greater abundance in SMP from CLV, but it must be noted that ethanol is not very odour active (Kilcawley et al. 2018). The ketone content of bovine milk has also been shown to be significantly affected by diet (Stefanon and Procida, 2004), with ketones derived from carbohydrate metabolism higher in the feed with greater levels of carbohydrate, such as in TMR. Acetoin (sweet, buttery, creamy), a primarily product of hetero-lactic fermentation combined with citrate formation by lactic acid bacteria (Wilkinson & Kilcawley, 2007), were positively correlated with TMR samples. Each feeding system had a significant effect on the concentrations of acetoin (TMR > GRS > CLV) and it was found to be the most discriminatory compound based on abundance levels between the diets by VIP (Figure.2.5), as determined by PLS-DA. Under high intake of fermentable carbohydrates (i.e., starch or non-starch soluble carbohydrates) in cow feeding diets, *Streptococcus bovis* and *Bifidobacterium spp.* undergo homolactic fermentation of lactose to produce acetic and lactic acid in the cow rumen (Hernandez et al. 2008). The combination of lactose homolactic fermentation and citrate fermentation provides the foundational mechanism for the synthesis of diacetyl and acetoin (Coolbear, Wilkinson & Weimer, 2011). As we did not undertake olfactory analysis or quantification we cannot assess its sensory impact but acetoin has an intermediate odour threshold and has the potential to influence sensory perception. Volatile sulfur compounds are also potentially crucial due to their high odour intensities and are derived from methionine and cysteine by rumen microbes (Faulkner et al. 2018). Dimethyl sulfone was most abundant in the CLV-SMP and at lowest abundance in the TMR-SMP and this is in agreement with other studies which found higher levels of dimethyl sulfone in milk and cheese from cows on pasture diets (Coppa et al. 2011; Faulkner et al. 2018; Villeneuve et al. 2013).

p-Cresol is a rumen metabolite associated with the degradation of β-carotene (Agabriel et al. 2004) and has been found at higher levels in pasture-derived dairy products (Faulkner et al. 2018; O'Callaghan et al. 2018). Lopez and Lindsay (1993) noted that the p-cresol has a "barn-yard" like odor and is responsible for "cowy flavor". Although p-cresol was not detected in SMP in this study, USA panels (Figure 2.3) identified a "barnyard flavor" in SMP from GRS and CLV which was not present in TMR SMP. Thus, it appears that p-cresol may be present below levels of detection by HS-SPME GCMS, but above its odor threshold.

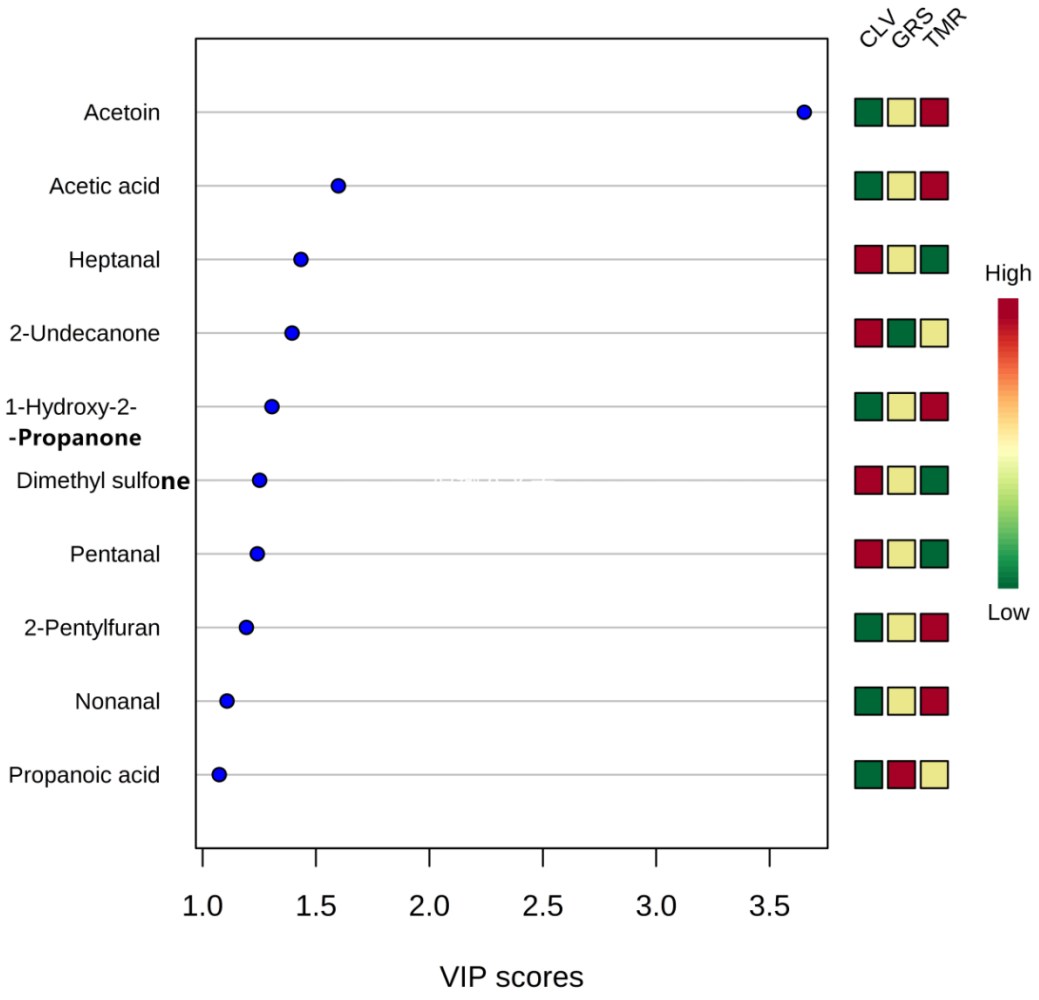


Figure 2.5 Variable Importance in Projection (VIP) plot shows the relevance of the most significant volatiles responsible for the differentiation between the SMP produced from different feeding regimes (CLV, GRS and TMR) samples, as determined by PLS-DA.
CLV = grass/clover, GRS = grass, and TMR = total mixed ration.

2.4 Conclusion

This study investigated the sensory perception of SMP produced by three separate feedings systems in a cross-cultural context between Irish, USA and Chinese consumers, trained Irish and Chinese assessors, and a trained USA sensory panel. Significant cross-cultural differences were evident in the perception of the SMP based on diet between the different cultural groups, and diet also influenced the volatile profile of SMP. Chinese consumers could not discern a difference between the CLV, GRS and TMR SMP, but rated "aftertaste liking", "aftertaste intensity" and "mouthfeel/thickness liking" differently than USA and Irish consumers. In general, USA consumers had preference for TMR SMP, but did not find many differences between CLV and GRS SMP. Irish consumers had lower scores for many attributes than both Chinese and USA consumers, but generally preferred CLV and GRS SMP than TMR SMP. ODP analysis by trained Chinese and Irish assessors found they could more easily discern that TMR SMP was different to CLV and GRS SMP. In general Irish assessors rated many positive attributes higher, and negative attributes lower in CLV and GRS SMP than in TMR SMP, although they only gave a negative association for "creamy aroma" in TMR SMP. Chinese assessors did not find any significant differences in 11 of the 22 attributes evaluated, but rated TMR negatively for "creamy aroma" and "creamy flavour" and positively for "painty aroma", "oxidised flavour" and "rancid butter". Chinese assessors did find a positive association with "creamy aroma", "cooked aroma", "painty aroma", "sour taste", and "rancid butter" for CLV SMP in comparison to GRS SMP, and a negative association for "sweet taste", "dairy sweet taste", and "carmelized flavour" for both CLV and GRS SMP in comparison to TMR SMP. Trained descriptive USA panelists found that TMR SMP was more "sweet aromatic", with less "grassy/hay" attributes than CLV or GRS SMP and that GRS

SMP had more “salty taste” than CLV or TMR SMP. The CLV SMP had a “cardboard/wet paper” attribute and both CLV and GRS SMP had a “cowy/barny” attribute which were absent in TMR SMP. Ten volatile compounds differed statistically based on diet, with acetoin derived from carbohydrate metabolism having the greatest impact based on abundance levels, and was significantly higher in TMR SMP than in CLV or GRS SMP.

This study has highlighted significant cross cultural sensory differences in SMP produced from pasture (CLV and GRS) and non-pasture (TMR) feeding systems, which are likely applicable to other dairy products produced from similar feeding regimes. It also highlighted differences between consumers and trained panellists, that were most apparent between Chinese consumers and Chinese trained assessors. Some of the main volatiles most likely responsible for these differences were also identified.

The study has highlighted the importance of product familiarity, as USA and Irish consumers, trained assessors and trained panelists preferences were essentially aligned with their familiarity to dairy products from non-pasture and pasture feeding regimes, respectively. Chinese consumers were less discerning in relation to diet, but both Chinese consumers and trained assessors scored many attributes quite differently than their USA or Irish counterparts, likely reflecting a lack of familiarity with dairy products in general.

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Chapter 3. Volatile profiling and cross cultural sensory assessment of yoghurt

Abstract

Understanding potential cross-cultural sensory differences in the perception of yoghurt products is important for export markets. This study investigated the impact of starter cultures on the volatile profile and cross cultural sensory perception of yoghurts produced from milk, whole milk powder and skim milk powder derived from a pasture based milk production system in Ireland and Germany. Yoghurt products were produced with different commercial starter cultures. Yoghurt samples were evaluated by Irish (n = 110) and German (n = 82) consumers using a sensory acceptance test (hedonic attribute testing) and by optimized descriptive profiling by Irish (n = 25) and German (n = 16) assessors familiar with dairy products. Volatile analysis was undertaken by headspace solid phase microextraction gas chromatography mass spectrometery. The abundance of 17 volatile compounds differed significantly between the samples and 6 of the 10 most influential were associated with lipid oxidation. Overall, there was no significant difference between three yoghurt products among German and Irish consumers, although some cross-cultural preferences were evident. Irish consumers rated the one sample (A) significantly higher for 'liking of texture' ($p<0.01$) and two others (B and C) significantly higher for 'liking of flavour' ($p<0.01$) than Germany consumers. Germany assessors found it more difficult to discern differences between the three yoghurt samples than Irish assessors, which may be related to a greater familiarity of Irish assessors with dairy products derived from a pasture based milk production system than German assessors. Some volatile and sensory associations were also evident; 'rancid butter flavour' and 'oxidised flavour' were correlated with 2-heptanone, 'astringency' with acetoin and 'creamy aroma' and 'sweet aroma' with benzaldehyde.

Keywords: Cross-cultural sensory, Yoghurt, Starter cultures, Volatiles

3.1 Introduction

Yoghurt is one of the most popular fermented milk products, and has received heightened interest in recent years, particularly with the advent of the use of probiotics in dairy products (Tamime and Robinson, 1999). Increased yoghurt consumption is attributed to the perceived health benefits and its consumer appeal (Madhu et al. 2012; Yüksel and Bakırcı 2015). The US Food and Drug Administration defines yogurt as, 'the food produced by culturing one or more of the optional dairy ingredients cream, milk, partially skimmed milk or skimmed milk, used alone or in combination with a characterizing bacterial culture that contains the lactic acid producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (US Food and Drug Administration, 2008). Typically, milk used for yoghurt production is fortified with dairy powders, usually skim milk powder or milk protein concentrate (Remeuf et al. 2003). Yoghurt is usually produced using mixtures of homofermentative lactic acid bacteria (LAB) such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* as the starter culture (Muramalla and Aryana, 2011; Kaneko et al. 2014). Lactic acid influences the physicochemical properties of casein micelles, allowing casein molecules to aggregate through hydrophobic and electrostatic interactions at the isoelectric point of casein (pH 4.6), giving yoghurt its characteristic texture (Lucey, 2004). The overall flavour of any fermented milk product is formed by a large number of volatile aromatic compounds during production. Carbonyl compounds and organic acids such as acetaldehyde, diacetyl, and acetic acid play an important role and can be utilised to evaluate the flavour quality of yoghurts (Zha et al. 2015). Ott et al. (1997) documented that acetaldehyde provided yoghurt with its characteristic flavour. Moreover, Erkus et al. (2013) reported that diacetyl also provides the uniquely sour buttery flavour to yoghurt.

In terms of cross-cultural differences in food preference, studies have demonstrated the significance of familiarity in influencing food preference. Different cultural food environments and dietary experiences impact sensory perception and consumer preferences (Prescott and Bell, 1995). A recent study by Garvey et al. (2020) demonstrated that familiarity contributed to sensory differences in Irish butter as perceived by German, Irish and USA consumers and assessors. Hay et al. (2021) also reported consumer sensory preferences for drinkable yoghurt and the impact of provenance was correlated with culture and familiarity between Chinese, European and New Zealand consumers. In another study, French trained panellists used twice as many attributes to describe soy yoghurt than Vietnamese panellists which was thought to be mainly due to the fact that the Vietnamese panellists were more familiar with the product (Tu et al. 2010). Moreover, Cheng et al. (2020) documented that Chinese consumers and trained assessors scored many skim milk powder attributes quite differently than their Irish or USA counterparts, which again was attributed to a lack of familiarity with dairy products, amongst the Chinese group. Therefore, the understanding of dairy product familiarity by consumers and cultural differences associated with food perception is essential in sensory research to understand differences in consumer behaviour.

The objective of this study was to investigate the volatile profile of yoghurts prepared with different starter cultures by headspace solid phase micro-extraction gas chromatography mass spectrometry (HS-SPME-GC-MS) and the sensory perception by consumers and untrained sensory assessors in Ireland and Germany.

3.2 Materials and Methods

3.2.1 Yoghurt Manufacture

Bovine milk, whole milk powder and skim milk powder were used as the main dairy ingredients for yoghurt manufacture. Raw milk was obtained from a local dairy company (Dairygold Co Operative Society Ltd, Cork, Ireland) and mixed with 9% (w/v) whole milk powder (moisture 2.7%, protein 35.4%, fat 26.5%) and 6% (w/v) skim milk powder (moisture 3.3%, protein 34.0%, fat 1.0%) (Dairygold Co Operative Society Ltd, Cork) and RO water to standard composition (5.24% protein, 2.44% fat, 6.5% lactose and 14.56% total solids). This mixture was homogenized using a GEA Niro Soavi S.p.A. Type: NS2006H non-aseptic homogeniser using 2- stage homogenization at 20,000 to 5,000 kPa. It was subsequently heat-treated using a Microthermics unit (UHT/HTST Electric Model 25HV Hybrid, Liquid Technologies, Wexford, Ireland) to 95 ± 2 °C and held for 5min, then cooled to 42 ± 2 °C and aseptically filled into three sterile containers (250L). Each milk was inoculated with three different commercial yoghurt starter cultures; A (YoFlex FD-DVS Mild 1.0 *Lactobacillus delbrueckii subsp. bulgaricus* , *Streptococcus thermophiles*, Chr. Hansen Ireland Ltd, Cork, Ireland) inoculated at 50 Units / 250 L; B (ABT-5 *Streptococcus thermophilus*, *Lactobacillus acidophilus-LA 5*, and *Bifidobacterium BB-12*, Chr. Hansen Ireland Ltd, Cork, Ireland) also inoculated at 50 Units / 250 L, and C (a 1:1 mixture of A+B) each at 50% of the original dose rate). After yoghurt inoculation, each yoghurt sample was immediately cooled and transferred at 4 °C to the sterile product outlet and aseptically packed into sterile 500 mL polypropylene sample bottles with screw cap. Sensory analysis was performed immediately after pasteurisation within two week. Samples were stored at 4 °C prior to any analysis that was not performed immediately.

3.2.2 Consumer Acceptance Testing

Consumers residing in Germany, (n=82), and Ireland (n=110) having similar characteristics in terms of gender (Ratio M/F, 30/70), occupation (student) and age (18-30 years), participated in the sensory acceptance study (hedonic attribute testing). Consumers were regular self-reported consumers of milk/yoghurt, had experiences in drinking powdered milk products, and were non-rejecters of milk. All sensory analysis was undertaken at the sensory facility within the School of Food and Nutritional Science, University College Cork, Co. Cork, Ireland and University of Applied Sciences, Muenster, Germany, according to International Standards (ISO 11136, 2014).

Samples were taken from the refrigerator (4°C) and left to stand at 12°C for 15 min prior to evaluation in 30 mL styrofoam tumblers. Sample presentation order was randomised (MacFie et al. 1989) and with samples presented with randomly generated three digit codes. The appearance of each sample was scaled using a 1–10 hedonic scale, where 1 = dislike extremely and 9 = like extremely (Supplementary Table 3.1). Consumer were initially asked to assess the colour 'colour-liking' using a 9-point hedonic scale. Then assessors were asked to assess for the 'liking of flavour', 'liking of texture', 'liking of appearance', 'liking of aroma', and 'overall acceptability'. Sensory data was collected on paper ballots in Ireland and by computerized data entry in Germany.

Supplementary Table 3.1 Sensory terms for the affective (consumer acceptance testing) and optimized descriptive profiling (ODP) of yoghurt

Descriptor	Explanation	Scale
Consumer Acceptance Testing		
Appearance-Liking	The liking of appearance	0 = extremely dislike 10 = extremely like
Flavour-Liking	The liking of flavour	0 = extremely dislike 10 = extremely like
Aroma-Liking	The liking of aroma	0 = extremely dislike 10 = extremely like
Texture-Liking	The liking of texture	0 = extremely dislike 10 = extremely like
Overall acceptability	The acceptability of the product	0=extremely unacceptable 10 = extremely acceptable
Colour-Liking	The liking of colour	0 = extremely dislike 10 = extremely like
Optimized Descriptive Profiling		
Appearance-colour	Appearance-Ivory to orange colour	0 = Pale, 10 = Yellow
Sweet aroma	The smell associated with dairy sweet milky products	0 = none, 10 = extreme
Creamy aroma	The smell associated with creamy/milky products	0 = none, 10 = extreme
Cooked aroma	The smell associated with cooked milk products	0 = none, 10 = extreme
Oxidised aroma	The smell associated with rancid or oxidised products	0 = none, 10 = extreme
Painty aroma	The smell associated with rancid paint type notes	0 = none, 10 = extreme

Supplementary Table 3.1 Cont.

Chalky Texture	Chalk like texture in the mouth	0 = none, 10 = extreme
Powdery Texture	Powdery texture in the mouth	0 = none, 10 = extreme
Viscosity	Thick texture in the mouth	0 = none, 10 = extreme
Sweet taste	Fundamental taste sensation of which sucrose is typical	0 = none, 10 = extreme
Cream flavour	The flavour associated with creamy/milky products	0 = none, 10 = extreme
Dairy sweet flavour	The flavours associated with sweetened cultured dairy products such as fruit yoghurt	0 = none, 10 = extreme
Dairy fat flavour	Intensity of fat flavour	0 = none, 10 = extreme
Oxidised flavour	The flavour associated with rancid or oxidised products	0 = none, 10 = extreme
Rancid butter	The flavour associated with rancid or oxidised butter	0 = none, 10 = extreme
Painty Flavour	The flavour associated with rancid paint type notes	0 = none, 10 = extreme
Fruity/Ester flavour	The flavours associated with fatty acid ethyl esters	0 = none, 10 = extreme
Cooked flavour	The flavour associated with cooked milk products	0 = none, 10 = extreme
Off-flavour	Off-flavour (Rancid)	0 = none, 10 = extreme
Astringent after-taste	Fundamental taste sensation of which aluminium sulphate is typical	0 = none, 10 = extreme

3.2.3 Optimized Descriptive Profiling

Optimized Descriptive Profiling (ODP) (Silva et al., 2012, 2013; Silva et al., 2014) was also undertaken in Germany and Ireland. Assessors were trained (O'Sullivan, 2016) in University of Applied Sciences, Muenster, Germany, Germany (n = 16) and in University College Cork, Cork, Ireland (n = 25). These assessors were presented with all samples simultaneously but with randomised order to prevent first order and carry-over effects (MacFie et al. 1989). Assessors used the consensus list of sensory descriptors which were measured on a 10 cm line scale with the term 'none' used as the anchor point for the 0 cm end of the scale and 'extreme' for the 10 cm end of the scale (Supplementary Table 3.1). For this study training and the use of a consensus sensory lexicon were used as described by Richter et al. (2010) and Cheng et al. (2020) for a Ranking Descriptive Analysis technique. Sensory terms, which were the main sensory dimensions, were pre-selected from the sample set using an expert sensory panel (n = 10). Assessors evaluated the intensity of each attribute for each sample on the scales. Attributes were presented along with the table describing the sensory terms (Supplementary Table 3.1). All samples were prepared in the same manner as the consumer analysis study and presented in triplicate.

3.2.4 Volatile Analysis

The yoghurt samples were analysed by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME GCMS) in triplicate. The yoghurt samples (4g) were weighed into 20 ml amber screw capped headspace vial (Apex Scientific, Maynooth, Co. Kildare) and equilibrated to 40 °C for 10 min with pulsed agitation of 5 sec at 500 rpm using a Shimadzu AOC 5000 Autosampler (Mason Technology Ltd, Dublin, Ireland). A single SPME fibre 50/30 µm carboxen/divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS), (Agilent

Technologies Ireland Ltd, Cork, Ireland) was exposed to the headspace above the samples in the vial for 20 min at a depth of 1 cm at 40°C. The fibre was retracted and injected into the gas chromatograph inlet and desorbed for 2 min at 250 °C. The fibre was conditioned and cleaned between runs using a bakeout conditioning station (Agilent Technology Ltd, Ireland) as per manufactures instructions. The blank vials was also analyzed at the start and end of each to ensure that no carryover occurred. Injections were made on a Shimadzu 2010 Plus GC (Mason Technology Ltd, Dublin) with an DB-624 UI (60 m x 0.32 mm x 1.80 μm) (Agilent Technology Ltd, Ireland) column using a split/splitless injector in splitless mode with a merlin micro seal (Agilent Technology Ltd, Ireland). The temperature of the column oven was set at 40°C, held for 5 min, increased at 5°C min^{-1} to 230°C then increased at 15°C min^{-1} to 260°C, yielding at total run time of 65 min. The carrier gas was helium held at a constant flow of 1.2 ml min^{-1}. The detector was a Shimadzu TQ8030 mass spectrometer detector (Mason Technology Ltd, Ireland), ran in single quad mode. The ion source temperature was 220°C and the interface temperature was set at 260°C. The mass spectrometer mode was electronic ionization (70v) with the mass range scanned between 35 and 250 amu. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, a commercial flavour and fragrance library (FFNSC Shimadzu, Mason Technology Ltd, Ireland) and an in-house library created in Shimadzu GCMS Solutions software (Mason Technology Ltd, Ireland) with target and qualifier ions and linear retention indices for each compound. Linear retention indices were performed as per the method of Van Den Dool and Kratz (1963). Spectral deconvolution was also performed to confirm the identification of compounds using AMDIS. Ten ul of a standard solution (1-butanol, dimethyl disulphide, butyl acetate, cyclohexane, benzaldehyde) at 10 mg kg^{-1} and 2-phenylethanol at 50 mg kg^{-1} in

methanol: water (1:99) were run before and after every series of samples to ensure that both the SPME extraction and MS detection were performing within specification. An auto-tune of the GCMS was carried out prior to the analysis to ensure optimal performance. Every sample was analysed in triplicate. Results were expressed as abundance values only which represent peak areas for individually identified volatile compounds, due to the difficulty of quantification using HS-SPME where multiple chemical classes are present.

3.2.4 Volatile Analysis

The statistical analysis of the volatile compounds was tested with KW-Partial Least Squares regression (KW-PLSR). PSLR combines features of Principal Component Analysis (PCA) and multiple regressions and is performed separately on a set of dependent variables from a large set of independent variables. Based on the PSLR Beta coefficients results, the compound which has a significantly higher ($p<0.05$) absolute value of the beta coefficient (Arjo, 2009) was used in the Kruskal-Wallis H test (one-way ANOVA on ranks) to evaluate the significant differences for each volatile compound amongst the yoghurt samples. To classify the yoghurt samples in a supervised multivariate model, partial least squares discriminant analysis (PLS-DA) was performed. The Variable Importance Plot (VIP) scores summarised the individual X variables and have an influence on the PLS model and rank in terms of importance on the Y axis (with variables of the highest importance at the top) (Strobl et al. 2007). VIP scores give a measure useful to show the volatile compounds' latent variables that contribute most to the underlying variation in different yoghurt starter cultures. Unsupervised hierarchical clustering analysis was used to show the patterns in the volatile data and is presented as a Heatmap. Consumer acceptance data obtained from the sensory trials were evaluated by one-way ANOVA using cultural differences

of perception and yoghurt starter cultures as primary factors. The post hoc Tukey test was performed to find which yoghurt were significantly different from a cross cultural (Ireland, German) consumer assessment. The SPSS V23.0 (IBM Statistics Inc., Armonk NY) was used for Kruskal-Wallis H test and one-way ANOVA. Spectrum descriptive Data were evaluated by analysis of variance with means separation (SAS version 9.2, Cary, North Carolina, USA). The Unscrambler X software, version 10.3 (Camo Software, Oslo, Norway) was used for PSLR analysis of milk powders data in different feeding systems and variance of ODP sensory data. Regression coefficients were analysed by Jack-knifing to derive significant indicators for the relationships determined in the quantitative APLSR (data not shown). MetaboAnalyst 4.0 (McGill University, Montreal, QC, Canada) software was used to perform the Variable importance plot (VIP), PLS-DA and Hierarchical clustering analysis (HCA) (Chong et al. 2020).

3.3 Results and Discussion

3.3.1 Sensory Evaluation

3.3.1.1 Irish, Germany Consumer Acceptance Testing

The mean scores for the hedonic attributes for Irish and Germany consumers for each yoghurt type are shown in Table 3.1. Some significant cross-cultural differences were evident in the perception of the three different yoghurt samples (A, B and C) and between both cultural groups (Germany and Ireland). Irish consumers rated all yoghurt samples statistically higher for ‘liking of appearance’ ($p<0.05$), and for ‘overall acceptability’ ($p<0.05$ for A and B and $p<0.01$ for C) than the German consumers. Irish consumers only scored ‘liking of flavour’ significantly higher for A ($p<0.05$) and B ($p<0.01$) than German consumers. Irish consumers also scored ‘liking

of texture' significantly higher ($p<0.05$) in the A sample than the Germany consumers. Both Germany and Irish consumers could not discern any difference between the A, B and C samples for 'liking of aroma'. German consumers did not score any attribute significantly higher than the Irish consumers

3.3.1.2 Optimized Descriptive Profiling – Irish and Germany Trained Assessors

The significance (p-value) of average means for the ODP attributes described by Irish and German assessors for yoghurt from different start cultures (A, B and C) are illustrated in Table 3.2. Some significant cross-cultural differences were evident. Of the 22 different attributes evaluated 11 ('colour'($p<0.01$), 'sweet aroma'($p<0.01$), 'creamy aroma'($p<0.01$), 'cooked aroma'($p<0.01$), 'painty aroma'($p<0.01$) , 'powdery texture'($p<0.01$), 'viscosity'($p<0.05$), 'salty taste'($p<0.01$), 'dairy sweet flavour'($p<0.01$), 'cooked flavour'($p<0.01$) and 'astringency'($p<0.01$)) were found to statistically differ between both cultural groups (Irish and German). German assessors scored 'colour' 'cooked aroma', 'cooked flavour', and 'salty taste' ('salty taste' not discerned by German assessors in any sample) significantly lower ($p<0.01$) than Irish assessors for all three types of yoghurt, while German assessors scored 'dairy sweet flavour' and 'astringency' significantly higher ($p<0.01$) in all three types of yoghurt. German assessors also scored 'sweet aroma', 'creamy aroma' and 'powdery texture' significantly lower in all three types of yoghurt than the Irish assessors, however the level of significance varied between yoghurt types. For example German assessors rated B significantly lower for 'sweet aroma' and 'creamy aroma' at $p<0.05$, and A significantly lower for these same attributes at $p<0.01$.

The ODP evaluation of each type of yoghurt is also shown in the APLSR plot (Figure 3.1). The yoghurt samples were subdivided into different clusters by Irish and

German assessors. Overall it was apparent that the German assessors could not discern differences between the different yoghurt samples, but that the Irish assessors could. The inability of the German assessors to distinguish any major sensory differences between the different yoghurt samples, may relate to overriding differences due to lack of familiarity with yoghurts produced from Irish pasture-fed milk, as so many specific attributes were deemed to be statistically similar, yet quite different to that discerned by the Irish assessors (Table 3.2). Something similar was found in a recent study by Ojeda et al. (2021) where Finnish consumers and assessors were better able to differentiate the quality level of Finnish cheese than non-Finnish cheese due to a lack of familiarity with non-Finnish cheeses.

Table 3.1 The mean scores of consumer acceptance analysis of yoghurt from Irish and Germany consumers.

Yoghurt starter cultures	A			B			C		
	Irish	German		Irish	German		Irish	German	
Sensory attributes			p-value			p-value			p-value
Liking of appearance	7.0 [a]	5.0[b]	***	7.3[a]	5.3[b]	***	7.3[a]	5.3[b]	***
Liking of aroma	5.6	5.1	NS	5.3	5.0	NS	5.4	5.3	NS
Liking of flavour	5.7[a]	4.6[b]	***	5.8[a]	5.0[b]	*	5.5	5.4	NS
Liking of texture	6.0[a]	4.8[b]	***	5.6	5.4	NS	5.8	5.4	NS
Overall acceptability	6.2[a]	4.7[b]	***	6.0[a]	5.0 [b]	***	6.0[a]	5.2[b]	*

Liking attributes were scored on a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely.

One-way ANOVA statistical analysis:* and *** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively .Values in the same row not sharing the same superscript (a,b) specify significant difference in sensory score carried out by Tukey post hoc test.

Table 3.2 Cross-cultural comparison of ODP evaluation by Irish and German assessors of yoghurt with different starter culture on A, B and C

	A			B			C		
	Irish	German	p-value	Irish	German	p-value	Irish	German	p-value
Colour	4.5[a]	3.8[b]	***	5.1[a]	3.8[b]	***	5.4[a]	3.8[b]	***
Sweet aroma	3.6[a]	2.4[b]	***	3.5[a]	3.0[b]	*	3.9[a]	2.6[b]	***
Creamy aroma	4.3[a]	3.2[b]	***	4.4[a]	4.0[b]	*	4.6[a]	3.9[b]	*
Cooked aroma	2.9[a]	1.8[b]	***	3.0[a]	2.0[b]	***	3.0[a]	2.0[b]	***
Oxidised aroma	2.7	2.3	NS	2.8	2.8	NS	2.6	2.4	NS
Painty aroma	2.4[a]	1.6[b]	***	2.8[a]	1.6[b]	***	2.6[a]	1.9[b]	***
Chalky texture	3.2	2.9	NS	3.5	3.3	NS	3.2	3.1	NS
Powdery texture	3.4[a]	2.4[b]	***	3.8[a]	2.3[b]	***	3.6[a]	3.0[b]	*
Viscosity	3.4[a]	3.0[b]	*	4.6[a]	2.7[b]	***	3.8[a]	2.8[b]	***
Sweet taste	2.7	3.0	NS	3.2	3.2	NS	3.2	2.8	NS
Sour taste	5.1	4.8	NS	5.2	5.4	NS	5.0	5.3	NS
Salty taste	2.5	ND	***	2.6	ND	***	2.3	ND	***
Creamy flavour	3.7	3.8	NS	4.6	4.6	NS	4.6	5.0	NS
Dairy sweet flavour	3.0[b]	4.0[a]	***	3.3[b]	4.3[a]	***	3.2 [b]	4.0 [a]	***
Caramelised	2.7	2.5	NS	2.7	3.1	NS	2.7	3.2	NS
Oxidised flavour	3.0	3.3	NS	2.6	2.9	NS	2.7	3.0	NS
Rancid butter flavour	2.6	2.9	NS	2.9	2.5	NS	2.5	2.5	NS
Painty flavour	2.5	2.1	NS	2.6	2.2	NS	2.5	2.2	NS
Grassy/hay flavour	2.0	1.9	NS	2.2	1.9	NS	2.0	1.7	NS
Cooked flavour	2.2[a]	1.3[b]	***	3.0[a]	1.5[b]	***	2.5[a]	1.6[b]	***
Off flavour	3.3	ND	***	3.3	ND	***	3.3	ND	***
Astringency	2.9[b]	3.9[a]	***	2.8[b]	4.5[a]	***	2.6[b]	3.9[a]	***

ANOVA values are the average results. One-way ANOVA statistical analysis:* and *** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively. Cross-cultural comparison: values in the same row not sharing the same superscript (a, b) indicate significant difference by one-way ANOVA (Tukey post hoc test).

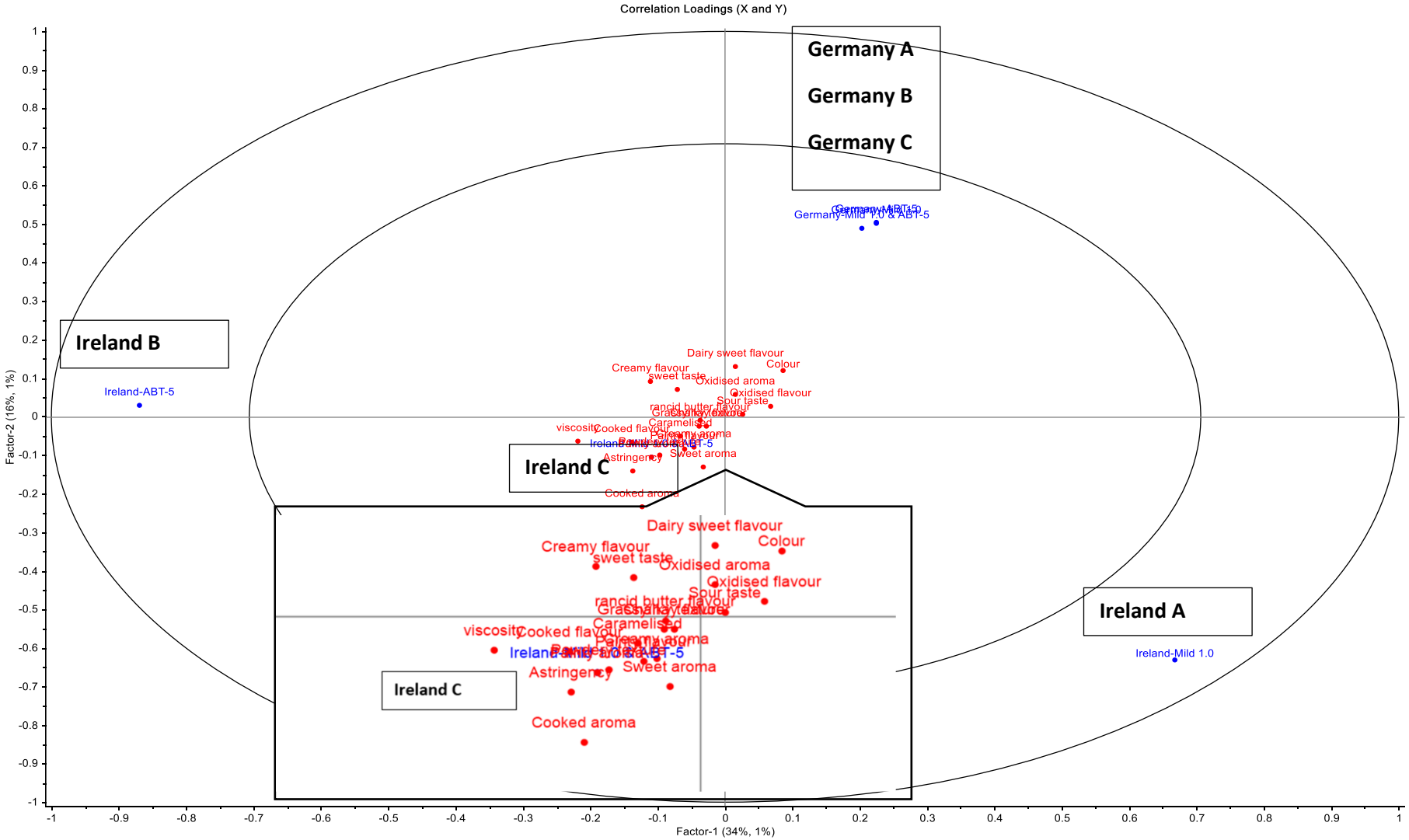

Correlation Loadings (X and Y)
Germany A
Germany B
Germany C
Germany-Mild 1.0 & ABT-5
Ireland B
Ireland-ABT-5
Ireland C
Ireland A
Ireland-Mild 1.0
Dairy sweet flavour
Colour
Creamy flavour
sweet taste
Oxidised aroma
Oxidised flavour
Sour taste
rancid butter flavour
Caramelised
viscosity
Cooked flavour
Sweet aroma
Astringency
Cooked aroma
Factor-2 (16%, 1%)
Factor-1 (34%, 1%)

Figure 3.1 Optimized Descriptive Profiling. ANOVA-Partial Least Squares regression (APLSR) plot for yoghurt with different starter culture on A, B or C by as evaluated Germany (n = 16) and Irish assessors (n = 25). The X-matrix = treatments*nationality (treatment evaluated in German or Ireland). The Y – matrix = sensory variables.

3.3.2 Volatile Analysis

In total, 24 volatile compounds were identified in these yoghurt samples (Table 3), consisting of aldehydes (9), hydrocarbons (3), ketones (11), sulphur (1) and a terpene (1). The abundance of 17 volatile compounds were statistically different between the 3 yoghurt samples (A, B and C). The abundance of the following volatiles; hexanal, benzaldehyde, acetone (2-propanone) and 2-octanone were statistically greater ($p<0.05$) in the A and B samples than in the C sample, as was pentanal, heptanal, octanal, nonanal, 2-nonenal-(E), undecanal, 2,4-decadienal and acetoin ($p<0.01$). Toluene, α-pinene ($p<0.05$), 2,3-butanedione and 2-hexanone ($p<0.01$) were significantly more abundant in A, than in B and C. 2-Butanone ($p<0.05$) and 2,3-pentanedione ($p<0.01$) were significantly more abundant in B and C, than in A. The differences in relation to abundance of VOC are clearly evident on the heatmap (Figure 3.2).

Table 3.3 Volatile compounds identified by head space solid phase micro-extraction gas chromatography mass spectrometry in yoghurt samples (A, B and C). Values indicate abundance values for each compound.

Name	CAS no.	LRI	Odor Descriptors	A	B	C	P-value
Aldehydes							
Pentanal	110-62-3	677	Pungent, almond like, sweet	4.82E+06 [a]	5.29E+06 [a]	3.73E+06 [b]	***
Hexanal	66-25-1	778	Green, slightly fruity, lemon, herbal	7.55E+06 [a]	7.28E+06 [a]	6.68E+06 [b]	*
Heptanal	111-71-7	875	Slightly fruity (balsam), fatty, sweet	5.40E+06 [a]	5.41E+06 [a]	4.20E+06 [b]	***
Benzaldehyde	100-52-7	955	Bitter, almond, burnt sugar	2.99E+06 [a]	2.97E+06 [a]	2.36E+06 [b]	*
Octanal	124-13-0	972	Waxy, citrus ,fruity	3.27E+06 [a]	3.23E+06 [a]	2.42E+06 [b]	***
Nonanal	124-19-6	1068	Green, citrus, fatty, floral, sweet	4.57E+06 [a]	4.62E+06 [a]	2.87E+06 [b]	***
2-Nonenal, (E)-	18829-56-6	1136	Cardboard , oxidized, fatty	3.00E+06 [a]	2.42E+06 [a]	1.82E+06 [b]	***
Undecanal	112-44-7	1260	Fruit, citrus, fatty	2.02E+06 [a]	1.56E+06 [a]	1.12E+06 [b]	***
2,4-Decadienal	2363-88-4	1295	Citrus, orange or grapefruit	2.02E+07 [a]	1.41E+07 [a]	9.25E+06 [b]	***
Hydrocarbons							
Benzene	71-43-2	628	Fruit, aromatic	3.10E+06	2.98E+06	2.84E+06	ns
Toluene	108-88-3	734	Paint, nutty, bitter, almond, Plastic	1.21E+07 [a]	1.13E+07 [b]	1.02E+07 [b]	*
p-Xylene	106-42-3	834	Not listed	4.34E+06	4.01E+06	3.90E+06	ns
Ketones							
Acetone	67-64-1	426	Sweet ,strong fruity, wood pulp, hay	1.92E+07 [a]	1.82E+07 [a]	1.58E+07 [b]	*
Diacetyl (2,3-Butanedione)	431-03-8	571	Buttery, creamy, vanilla	1.62E+08 [a]	7.04E+07 [b]	3.93E+07 [b]	***
2-Butanone	78-93-3	572	Buttery, sour milk, varnish-like	3.92E+06 [b]	4.36E+06 [a]	4.30E+06 [a]	*
2-Pentanone	107-87-9	672	Cheesy, sweet, fruity	8.08E+06	7.83E+06	7.70E+06	ns
2,3-Pentanedione	600-14-6	679	Buttery, vanilla, mild	4.48E+07 [b]	6.09E+07 [a]	6.27E+07 [a]	***
Acetoin	513-86-0	718	Buttery, mild ,creamy	6.27E+05 [a]	6.50E+05 [a]	3.79E+05 [b]	***
2-Hexanone	591-78-6	772	Floral, fruity	4.23E+06 [a]	3.47E+06 [b]	3.30E+06 [b]	***
2-Heptanone	110-43-0	868	Blue cheese, spicy, fruit	6.46E+07	6.78E+07	6.35E+07	ns

Table 3.3 Cont.

2-Heptanone, 4-methyl-	6137-06-0	912	Not listed	3.99E+06	3.78E+06	3.57E+06	ns
2-Octanone	111-13-7	962	Fruity, blue and parmesan cheese-like	2.61E+06 [a]	2.46E+06 [a]	2.29E+06 [b]	*
2-Undecanone	112-12-9	1249	Floral ,rose like	2.64E+06	2.63E+06	2.43E+06	ns
Sulphur							
Dimethyl sulfide	75-18-3	434	Corn like, lactone-like, sulphurous, cabbage	5.45E+06	5.93E+06	4.74E+06	ns
Terpene							
α -Pinene	80-56-8	884	Mint, pine oil	2.32E+06 [a]	1.55E+06 [b]	1.05E+06 [b]	*

RI: Retention index. REF RI: Reference retention index. CAS no: Chemical Abstracts Service Number. Kruskal-Walliss Ranking test statistical analysis:* and *** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively .Values in the same row not sharing the same superscript (a, b) specify significant difference in peak area value average. Odour descriptions are sourced from the following website: http://flavornet.org/flavornet.html and http://www.thegoods centscompany.com/

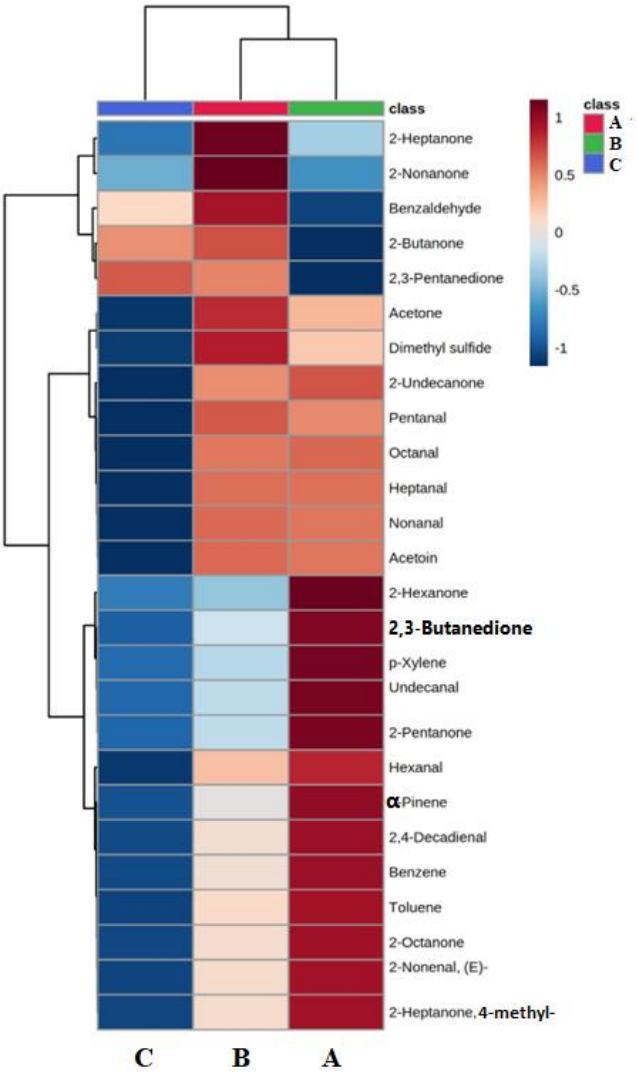


Figure 3.2 Hierarchical clustering analysis (heatmap) of volatile compounds derived from A, B and C. The degree of positive and negative correlation of is indicated by + 1 (red) to − 1 (blue). (For interpretation of the references to colour in this figure legend.

Variable importance coefficients (VIP) scores highlight those volatiles contributing most to the observed discrimination. Ten volatiles with a VIP $\geqslant$ 0.80 were responsible for the most discrimination between the 3 samples (Figure 3.3). These consisted of pentanal, nonanal, octanal, 2,4-decadienal, heptanal, 2,3-butanedione, α-pinene, 2-nonenal (E), acetone and acetoin. It is interesting to note that 6 of the 10 volatiles were aldehydes (pentanal, nonanal, octanal, 2,4-decadienal, heptanal and 2-nonenal (E)) derived from lipid oxidation. Pentanal ($P < 0.05$), nonanal ($P < 0.001$) and heptanal ($P < 0.001$) were most abundant in B and least so in C, while octanal ($P < 0.001$), 2,4-decadienal ($P < 0.001$) and 2-nonenal (E) ($P < 0.001$) were most abundant in A and least so in C, which were highest in B and lowest in C. Pentanal and 2,4-decadienal are derived from linoleic and arachidonic acid, and both nonanal and octanal are derived from oleic acid with heptanal derived from linoleic and oleic acid and 2-nonenal-(E) from linoleic acid (Clarke et al. 2022). It is difficult to understand how different starter cultures impact lipid oxidation in these yoghurt samples, except that different lipolytic activities may result in differences in abundances of free fatty acids that likely impact on the extent of lipid oxidation (Ajmal et al. 2018). The potential aromatic perception of these aldehydes like all volatiles is dependent upon their relative abundance and odour threshold (Chen et al. 2020). Most lipid oxidation aldehydes are associated with green, grassy aromas at lower concentrations, but are generally perceived as off-notes at higher concentrations (Clarke et al. 2020). Chen et al. (2017) provided aroma descriptors and odour thresholds (in water) for pentanal (fruit, bread, sweet) 0.022 mg kg^{-1}, nonanal (sweet, floral, citrus, grass-like) 0.04 mg/kg, octanal (fat, soap, lemon, green) 0.009 mg kg^{-1}, heptanal (green, sweet) 0.06-0.55 mg kg^{-1} and for 2-nonenal-(E) (green, fatty) 0.0004 mg kg^{-1} (Chen et al. 2017). 2,4-Decadienal has been described as fatty, oily, green, chicken skin-like, fried (Clarke

et al. 2020) with an odour threshold of ~2 mg kg^{-1} in oil (Garcia-Oliveira et al. 2021). This suggests that octanal pentanal and nonanal are the most odour active and therefore are the most likely to contribute to aroma perception.

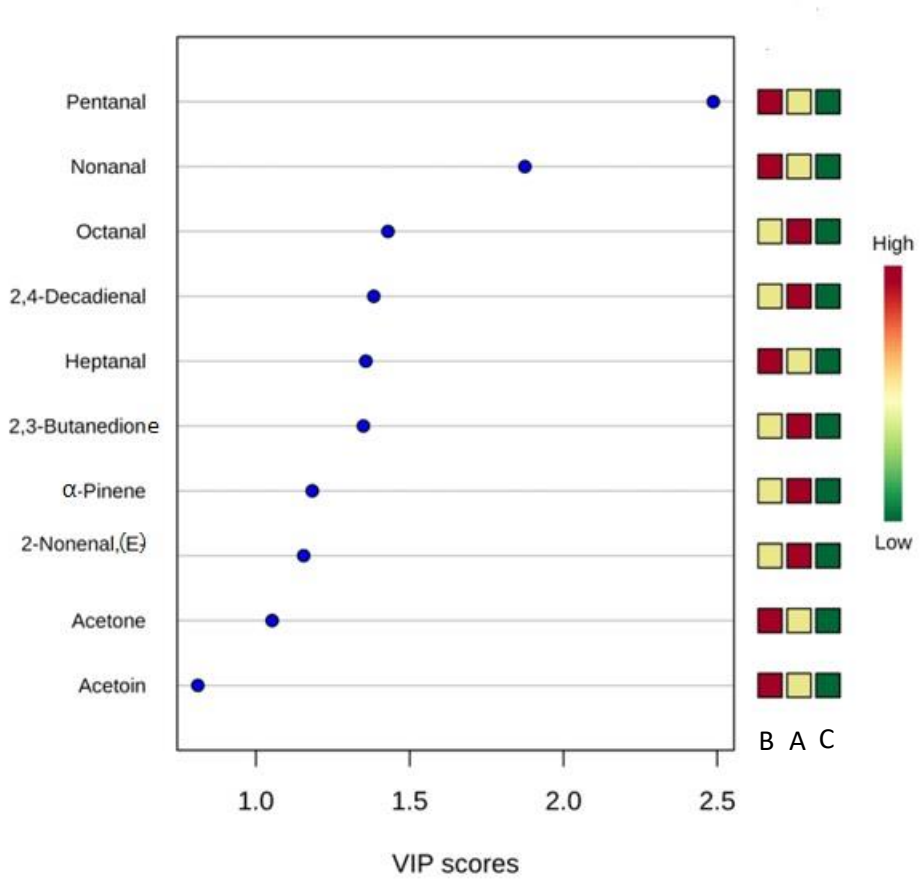


Figure 3.3 Variable Importance in Projection (VIP) plot shows the relevance of the most significant volatiles responsible for the differentiation between the yoghurt samples (A, B and C), as determined by Partial Least Squares- Discriminate Analysis (PLS-DA).

In fermented dairy products, various reviews have highlighted compounds with four carbon atoms are responsible for the typical aroma (butter-like) of yoghurt, include 2,3-butanedione (diacety), acetone (2-propanone) and acetoin (3-hydroxybutanone) (Ott et al. 1997; Chen et al. 2017; Routray and Mishra, 2011) and therefore the formation of these products is significantly dependent on the activities of the starter cultures utilized in their production, as they can be generated from glycolysis or citrate metabolism of several LAB (*Lactococcus, Leuconostoc*, and *Weissella species*) (Chen et al., 2017; Benozzi et al., 2015). The primary flavor compounds generated through the metabolic breakdown of citrate include acetate, diacetyl, acetoin, acetone, and 2-butanone. Diacetyl could be produced directly from enzymic action on acetaldehyde-thiamine pyrophosphate (TPP) and acetyl-CoA and the diacetyl synthase enzyme has never been identified clearly in LAB (McSweeney & Sousa, 2000). *S. thermophilus,* and *Lb. bulgaricus* strains used in this study produce diacetyl due to their high capacity to metabolise citrate (Chen et al. 2017; Passerini et al. 2013; Peng et al., 2022). Diacetyl ($P < 0.01$) was most abundant in A and lowest in C. Diacetyl (buttery, creamy, vanilla) is an intermediate metabolite, and its synthesis and decomposition are controlled by many pathways, has previously been identified as the most odour active volatile compound in yoghurt with an odour threshold of 0.0011 mg/kg in water (Chen et al. 2017; Zhang et al., 2015; Liu et al. 2022). Acetoin (buttery) is produced from α-acetolactate by the action of acetolactate decarboxylase and converted from 2,3-butanedione (Chen et al. 2017; Liu et al. 2022) and thus dependent upon its abundance. Acetoin ($P < 0.01$) was at greatest abundance in B and lowest in C. This study revealed that *Bifidobacterium* BB-12 might contributed to the formation of acetoin in yogurt. Wang et al. (2021) observed that co-culturing *Lactobacillus casei* and *Bifidobacterium animalis ssp* strains resulted in increased

production of diacetyl and acetoin, suggesting the possibility of metabolic interactions. The odour threshold of acetoin in water is 0.014 mg kg^{-1} (Cheng et al. 2017). Acetone (sweet, fruity) has an odour threshold of 0.832 mg kg^{-1} in water (Cheng et al. 2017). Acetone ($p < 0.05$) was also must abundant in B and least abundant in C. Even though acetone is thought to be produced by some LAB as its concentration has been shown to increase over fermentation, it is unclear how this can be achieved by lactic acid bacteria (Routray and Mishra, 2011). However, acetone can also be directly transferred from milk (Liu et a. 2022) and may also be generated by lipid oxidation (Fruehwirth et al. 2021). It is also worth mentioning that acetone has also been negatively correlated with yoghurt flavour (Cheng, 2010), and therefore its impact on flavour may be concentration dependent. 2,3-Pentanedione (buttery, caramellic, vanilla-like) has been suggested as the major endogenous odorant compounds of yogurt (Ott et al. 1997; Picon et al. 2008; Liu et al. 2022) and is derived from pyruvate metabolism. 2,3-Pentanedione ($p < 0.05$) was must abundant in B and C and least abundant in A. The synthesis of 2,3-pentanedione in yogurt fermentation was found to rely on threonine, pyruvate and acetate fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Ott et al. 2000; Martin et al., 2011).

Some other no citrate metabolism volatiles were also significantly different between the samples; undecanal, and 2-hexanone at $P< 0.05$, and hexanal, benzaldehyde, toluene, 2-butanone and 2-octanone at $P< 0.01$) (Table 3.3). The aldehydes hexanal (green, cut-grass) and undecanal (fatty) are also products of lipid oxidation and are often present in yoghurt (Cheng, 2010; Chen et al. 2017; Liu et al. 2022). Benzaldehyde (almond, burnt sugar) is also often found in yoghurt (Cheng et al. 2017) and is derived from aromatic amino acid catabolism but not thought to significantly contribute to yoghurt aroma (Zhang et al. 2020). Toluene (paint) is

derived from animal diet mainly as product of β-carotene metabolism in the rumen (Cheng et al. 2017; Kilcawley et al. 2018) and therefore difficult to discern why abundances may differ between these samples. In addition even though Cheng et al. (2017) stated an odour threshold of 0.024 mg/kg in water, it is generally noted as not very odour active in dairy products (Kilcawley et al. 2018). The methyl ketones 2-hexanone and 2-octanone are also products of oxidation of unsaturated fatty acids, followed by decarboxylation (Zhang et al. 2020). Chen et al. (2017) described 2-hexanone as floral, fruity with an odour threshold of 0.56 mg kg^{-1} in water. 2-Octanone is not common in yoghurt but has been noted to have an odour activity of 0.06 mg kg^{-1} in Chinese milk fan cheese (Tian et al. 2019), and a fruity, musty, unripe apple, green aroma (Qian and Burbank, 2007). 2-Butanone (Sweet, odour, butterscotch) is derived from carbohydrate metabolism (Marsili, 2016) and is suggested to also contributed to the aroma and flavour of yoghurt (Zaręba et al. 2014; Sfakianakis and Tzia, 2017; Cheng, 2010). Chen et al. (2017) noted 2-butanone has an odour threshold of 17–32 mg kg^{-1} in water. α–Pinene (herbal) is a terpene and has an odour threshold of 0.018 mg kg^{-1} in air (Pullen, 2007) and is not uncommon in many dairy products. Its presence in milk is related to the diet of the cow thus difficult to discern how abundance was greatest ($P < 0.05$) in A and least in C. However, some monoterpenes such as α-pinene may also be produced from the metabolism of sesquiterpenes and therefore may be a factor impacting on differences in these yoghurt samples (Kilcawley et al. 2018).

APLSR was conducted to study the relationships of the individual compounds with the sensory descriptors by Irish assessors (German assessors were unable to discern differences between three yoghurts). The X-matrix was composed of 24 compounds, whereas the Y-matrix was designated as the sensory attributes from ODP (Figure 3.4). When the two PCs were taken into account, 70% of the volatile variables

explained 33% of the variation among the sensory data and starter cultures. All variances were placed between the inner ($r^2 = 0.5$) and outer ellipses ($r^2 = 1.0$), thereby indicating the APLSR model can sufficiently described the associations between descriptors and VOCs. The centre ellipsoid in Figure 3.4(A) indicates 50% of the explained variation. Many volatile compounds were located outside the ellipsoid, which highlights that they make a considerable contribution to the model. Figure 3.4 demonstrates that the yoghurt samples are separated along PLS1, with the C samples on the left side, B in the centre and the A on right side. The C samples were also significantly correlated to 2,3-pentanedione and 2-butanone ($p<0.05$, obtained from the jack-knife uncertainty test). B samples were situated in the centre of the plot and appeared to be correlated most with astringency, acetoin and dimethyl sulfide. The A samples were located in the right portion of the diagram and positively correlated ($p<0.05$) with hexanal, heptanal, octanal, nonanal, (E)-2-nonenal, 2.4-decadienal, benzene, 4-methyl-2-heptanone, toluene, 2,3-butanedione, 2-octanone and α-pinene. Acetoin were positively correlated with astringency. This result is similar to that obtained by Soukoulis et al. (2010) who found polar flavor compounds in fermented milks (acetaldehyde, acetoin, diacetyl, acetic acid etc.) are the most prominent factors leading to perceived sourness and astringency. 2-Heptanone was positively correlated with rancid butter flavour and oxidised flavour, and has previously been found to be responsible for oxidized flavour (fatty and soapy) in concentrated milk (Li and Wang, 2016). Creamy aroma and sweet aroma were also positively correlated with benzaldehyde, which has been descried as the almond flavour in plant-based milk and may confer a sweeter taste for some beverages (Vaikma et al. 2021).

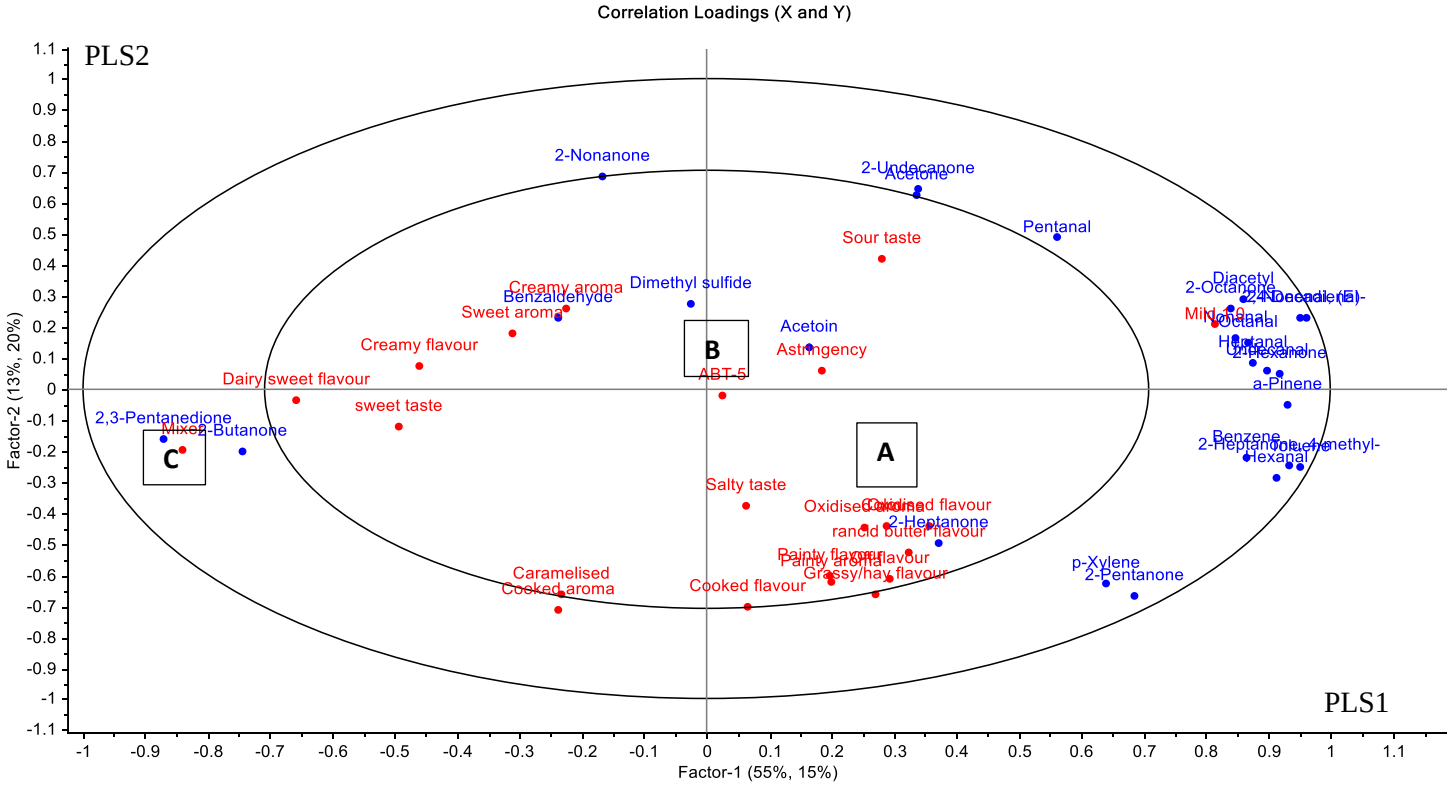
3.4 Conclusion

This study has demonstrated that significant cross-cultural differences were evident in the perception of the yoghurt produced from different starter cultures as assessed by Irish and German consumers and assessors. In general, Irish consumers gave higher scores for all yoghurt samples for 'liking of appearance', and 'overall acceptability' than Germany consumers. Irish consumers rated the A sample significant higher for 'liking of texture' and had higher scores for 'liking of flavour' for the A and B yoghurt sample. Overall, neither Irish nor German consumers could discern a difference between the three yoghurts in terms of their 'liking of appearance', 'liking of aroma', 'liking of texture', 'liking of flavour' and 'overall acceptability'. ODP analysis highlighted that Irish assessors could easily discern differences between the yoghurt samples, while German assessors did not. It seems plausible that the familiarity of Irish dairy products produced from milk derived from a pasture based feeding system plays a vital role in the acceptability and preferences of Irish consumers and assessors. German consumers and assessors are less familiar with yoghurt produced from this feeding system and therefore this may have impacted on their ability to discern differences. In general, Irish assessors rated 'colour, 'sweet aroma', 'cream aroma, 'cooked aroma', 'painty aroma', 'powdery texture' 'viscosity, 'salty taste', 'cooked flavour' and 'off flavour' higher in all samples than German assessors. However, German assessors scored 'dairy sweet flavour' and 'astringency' higher in all samples than the Irish assessors. Only Irish assessors had significantly higher preferences for 'viscosity' for the B sample than the A and C samples, while German assessors did not find any differences between the samples.

In total 24 VOC compounds were identified in these yoghurt samples and the abundance of 17 (~71%) were significantly different between the three yoghurt samples, with pentanal derived from linoleic and arachidonic acid having the greatest

impact based on abundance levels, which was significantly higher in A and B samples. The 10 most discriminating VOC between these yoghurt samples were all directly or indirectly associated with lipid oxidation (pentanal, nonanal, octanal, 2,4-decadienal, heptanal, 2,3-butanedione, α-pinene, 2-nonenal (E), acetone and acetoin). 2,3-Butanedione (diacety), acetone (2-propanone), acetoin (3-hydroxybutanone) and 2,3-pentanedione showed statistically significant among the three starters, due to the starters' different biosynthetic capacities for the formation of volatiles. However, many of these compounds remain unidentified and cannot be traced back to their metabolic precursors with current knowledge, due to the complex and intricate network of pathways involved in the formation of flavor compounds. In this study, even minor differences in the composition of a starter culture can significantly impact the aroma profile generated during fermentation. Advancements in functional annotation of key enzymes through genomics, proteomics, and metabolomics techniques, as well as the use of genome-scale metabolic models, have facilitated the development of novel approaches to comprehend, regulate, and direct the formation of aromas in dairy fermentation processes. 'Rancid butter flavour' and 'oxidised flavour ' were correlated with 2-heptanone. Acetoin was positively correlated with astringency and benzaldehyde was correlated to 'creamy aroma' and 'sweet aroma'.

(A)


Correlation Loadings (X and Y)
PLS2
PLS1
Factor-2 (13%, 20%)
Factor-1 (55%, 15%)
2-Nonanone
2-Undecanone
Acetone
Pentanal
Sour taste
Creamy aroma
Benzaldehyde
Dimethyl sulfide
Sweet aroma
Acetoin
Astringency
Creamy flavour
ABT-5
Dairy sweet flavour
sweet taste
2,3-Pentanedione
Mixed-Butanone
Salty taste
Caramelised
Cooked aroma
Cooked flavour
Grassy/hay flavour
2-Heptanone
p-Xylene
2-Pentanone
Diacetyl
2-Octanone
Octanal
Hexanal
2-Hexanone
a-Pinene
Benzene
Hexanal
A
B
C

(B)

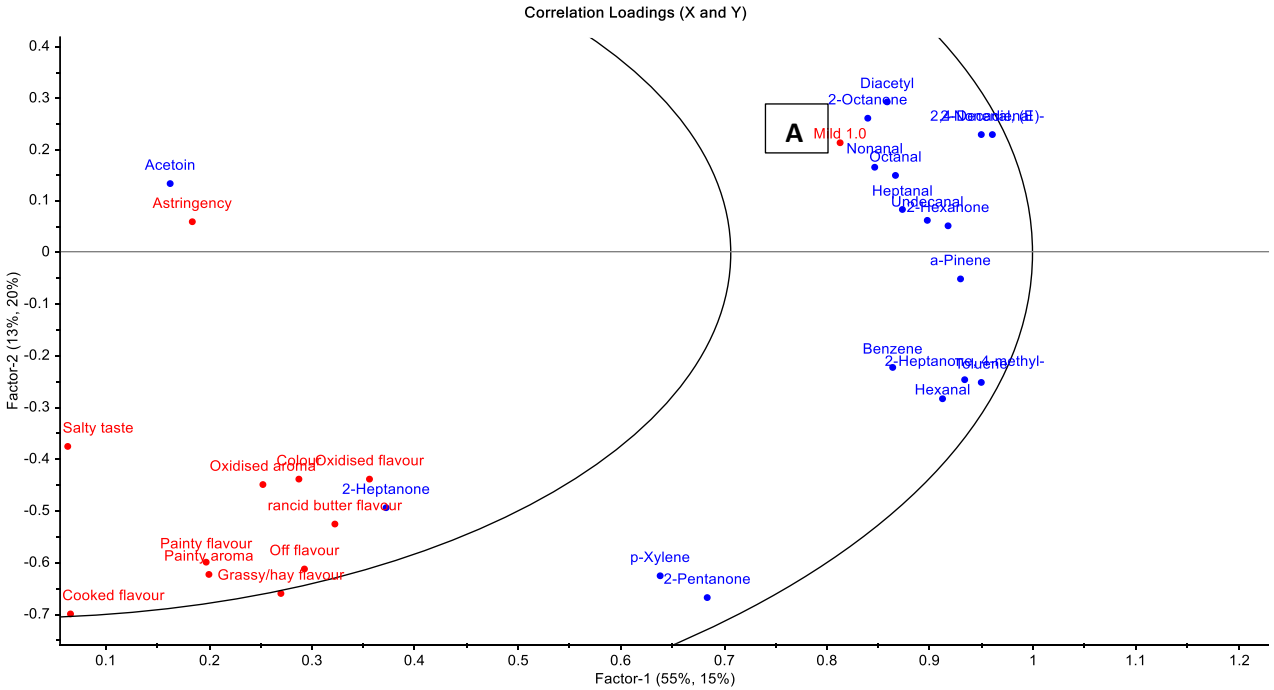

Correlation Loadings (X and Y)
Factor-2 (13%, 20%)
Factor-1 (55%, 15%)
A
Diacetyl
2-Octanone
Mild 1.0
Nonanal
Octanal
Heptanal
Undecanal
2-Hexanone
a-Pinene
Benzene
Toluene
Hexanal
Acetoin
Astringency
Salty taste
Oxidised aroma
Oxidised flavour
2-Heptanone
rancid butter flavour
Painty flavour
Painty aroma
Off flavour
Grassy/hay flavour
Cooked flavour
p-Xylene
2-Pentanone

Figure 3.4 (A)ANOVA-PLSR correlation loadings plot of sensory attributes (aroma and flavour) and volatile compounds(X-matrix) for three starter cultures (A,B and C) by Irish assessors. Ellipses represent r2 =0.5 and 1.0, respectively.(B)Enlargement of the ellipse.

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Chapter 4. Comparison of automated extraction techniques for volatile analysis of whole milk powder

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Abstract

Volatile profiling of whole milk powder is valuable for obtaining information on product quality, adulteration, legislation, shelf life, and aroma. For routine analysis, automated solventless volatile extraction techniques are favoured due their simplicity and versatility, however no single extraction technique can provide a complete volatile profile due to inherent chemical bias. This study was undertaken to compare and contrast the performance of headspace solid phase microextraction, thermal desorption, and HiSorb (a sorptive extraction technique in both headspace and direct immersion modes) for the volatile analysis of whole milk powder by gas chromatography mass spectrometry. Overall, 85 unique volatiles were recovered and identified, with 80 extracted and identified using a non-polar gas chromatography column, compared to 54 extracted, and identified using a polar gas chromatography column. The impact of salting out was minimal in comparison to gas chromatography column polarity and the differences between the extraction techniques. HiSorb extracted the most and greatest abundance of volatiles, but was heavily influenced by the number and volume of lactones extracted in comparison to the other techniques. HiSorb extracted significantly more volatiles by direct immersion than by headspace. The differences in volatile selectivity was evident between the techniques and highlights the importance of using multiple extraction techniques in order to obtain a more complete volatile profile. This study provides valuable information on the volatile composition of whole milk powder and on differences between extraction techniques under different conditions, which can be extrapolated to other food and beverages.

Keywords: whole milk powder, automated volatile extraction, gas chromatography mass spectrometry, HiSorb, headspace solid phase microextraction, thermal desorption

4.1 Introduction

The global production of whole milk powder (WMP) was 10.8 million tons in 2019 and is anticipated to reach 13.2 million tons by 2024 (Milk Powder Market, 2019). It remains a considerable export product for Ireland with 57,000 tons exported in 2019 (Ireland Dairy Sector, 2021). Dairy powders such as WMP have unique flavor characteristics that are heavily influenced by fat content and fat distribution (Park and Drake, 2014), but are also very susceptible to lipid oxidation (Clarke et al. 2019). Many studies on the volatile properties of dairy products have only evaluated single extraction techniques. However, as all extraction techniques have inherent bias towards certain volatiles based upon the properties of the volatile organic compounds (VOCs), their affinity to the sample matrix, and the properties and parameters of the extraction technique (Bertuzzi et al. 2018), it is therefore useful to evaluate a wider range of extraction techniques in order to get the best possible representative volatile profile of a sample.

Arguably the most widely used volatile extraction technique to date is solid phase microextraction (SPME), mainly due to its versatility, ease of use (as it is fully automatable), the wide range of coating materials available (single, dual, or multiple phases in different thicknesses), and its general robustness. It can be used as a direct immersion (DI) technique or, most commonly, as a headspace (HS) technique. Headspace solid phase microextraction (HS-SPME) is a static HS technique that has been extensively applied to analyze VOCs in dairy products, with the divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber finding the greatest use, due to its potential ability to capture a broader range of VOCs owing to the inherent properties of each phase (Tunick et al. 2013; Jelen et al. 2012; Heaven and Nash, 2012). However, the relatively limited surface capacity of the fiber can

result in competition between analytes for adsorption/absorbtion sites and displacement resulting in increased bias for certain VOCs (Mondello et al. 2005). Thermal desorption (TD) is a well-established dynamic extraction technique, where an inert carrier gas strips the volatiles from a sample where they are subsequently trapped in a sorbent packed tube with absorbent/adsorbent material (Materić et al. 2015; Horn et al. 2012). The main advantages are the wide range of sorbent phases available and the large capacity of sorbent phase. However, managing moisture can be problematic, and this may be why its use in dairy applications is limited (Jansson et al. 2014; Valero et al. 1997). Tenax (TEN) is typically the most widely used sorbent material in TD because of its affinity for VOCs with a very wide range of boiling points between 60 ◦C and 300 ◦C (Rabaud et al. 2002). Recently, another passive sorbent extraction technique was developed called HiSorb™ (Markes International Ltd., Llantrisant, UK). It is somewhat similar to stir bar sorptive extraction (SBSE) (Lancas et al. 2009) and can also be performed as a headspace (HS) or as a direct immersion (DI) technique. With HiSorb to date, a single sorbent phase polydimethylsiloxane (PDMS) is coated in a specially designed probe that can be either exposed to a HS above a sample or directly immersed in a liquid sample under controlled conditions. After exposure, the probe is placed in an empty sorbent tube and treated in a similar manner to a TD sorbent tube, where it is desorbed.

In terms of sample preparation, “salting out” is a useful practice to potentially increase the extraction efficiency of certain volatile analytes. Salt, usually sodium chloride (NaCl), is added to the sample, which reduces the solubility of hydrophobic compounds, resulting in decreased water availability and thus, in theory, making polar and low molecular weight VOCs easier to extract (Bertuzzi et al. 2017). The polarity of the gas chromatography (GC) column is also an important factor in relation to the

separation of individual VOCs. The most common types are polar and non-polar phases, both of which offer better separation and resolution for specific chemical classes (Imhof and Bosset, 1994) , with non-polar phases having greater stability. Therefore, in order to obtain the best possible volatile profile, it is also useful to assess both polar and non-polar GC columns.

In this study, we compared the ability of four automated volatile extraction techniques (HS-SPME, TD, and HiSorb as HS (HS-HiSorb) and as DI (DI-HiSorb)) for their ability to profile volatile compounds in WMP using gas chromatography mass spectrometry (GC-MS). Each extraction technique was assessed with or without salting out and using both a polar and non-polar GC column.

4.2 Materials and Methods

4.2.1 Preparation of Whole Milk Powder

Raw milk was produced from 54 Friesian cows at the Teagasc Moorepark dairy farm, Fermoy Co., Cork, Ireland. The milk was pre-heated to 50 ˚C in an APV plate heat exchanger (SPX Flow Technology, Crawley, West Sussex, UK), separated by a centrifugal disk separator, and pasteurized at 72 ˚C for 15 s. The pasteurized milk was subsequently preheated to 78 ◦C and evaporated in a Niro three-effect falling film evaporator (GEA Niro A/S, Soeborg, Denmark) at sequential effect temperatures of 73 ˚C, 64 ˚C, and 55 ˚C. Concentrate feed was introduced to a Niro Tall-Form Anhydro three-stage spray dryer (GEA Niro A/S, Soeborg, Denmark) (air inlet temperature = 180 ◦C and air outlet temperature = 85 ˚C) at approximately 43% total solids (TS) with a centrifugal atomizer (GEA Niro A/S, Soeborg, Denmark) at Moorepark Technology Ltd. (Fermoy Co., Cork, Ireland). Primary and secondary fluidized beds were maintained at 74 ˚C and 24 ˚C, respectively. Fines were returned to the cyclone to the top of the spray dryer. WMP samples were stored at room temperature in sealed 900

g aluminum vacuum cans until analysis. WMP samples were dissolved at 10% solids (w/v) using ultra-pure deionized water and stored at 4 ˚C overnight to ensure solubility, without overhead lights to prevent lightinduced off-flavor formation. Each extraction technique was assessed with or without salting out. NaCl (0.75 g) (Merck, Co., Wicklow, Ireland) was added to 5 mL of the 10% w/v WMP sample, equivalent to 15% NaCl w/v. This was mixed until soluble (~30 min).

4.2.2 Internal and External Standard Preparation

To monitor the performance of the GC-MS operating conditions, an external standard (ES) solution was added at the start and end of each GC-MS sample run. The ES was comprised of 1-butanol, dimethyl disulfide, butyl acetate, cyclohexanone, and benzaldehyde (Merck, Ireland) at 10 mg L^{-1} with 2-phenyl-D5-ethanol (Merck, Arklow, Co., Wicklow, Ireland) added at 5 mg L^{-1} in ultra-pure water. For the HS-SPME technique, 10 µL of ES was added to the sample in a 20 mL amber La-Pha-Pack HS vial with magnetic screw caps and a silicone/polytetraflurorethylene septa (Apex Scientific Ltd., Maynooth, Ireland); see details in Section 4.2.3.1. The ES (10 µL) was also added to the TD tube containing the sample extract for both TD and HiSorb (HS-HiSorb and DI-HiSorb), the details of which are described in Sections 4.2.3.2 and 4.2.3.3. To monitor the performance of each extraction procedure, an internal standard (IS) of 2-phenyl-D5-ethanol and 4-methyl-2-pentanol (Merck, Arklow, Co., Wicklow, Ireland) at 20 mg L^{-1} in ultra-pure water, was added (50 µL) to each WMP sample prior to extraction.

4.2.3 Extraction Procedures

The following codes were used to describe each extraction technique with and without salting out for both polar and non-polar GC columns (Table 4.1). An

extraction temperature of 40 ◦C was used for each technique based on previous experience and to ensure sufficient VOC extraction without creating additional VOC due to Maillard reactions or caramelization during the extraction process. The extraction times varied between techniques based on specific aspects of each technique and on previous experience. An equilibration step was necessary for the HS-SPME to maximize the VOC concentration in the HS prior to extraction.

Table 4.1 Codes used to describe each extraction technique

Code	Description
HS-SPME S	*Head space solid phase microextraction with salting out*
HS-SPME NS	*Head space solid phase microextraction without salting out*
TD S	*Thermal desorption with salting out*
TD NS	*Thermal desorption without salting out*
DI-HiSorb S	*Direct Immersion HiSorb with salting out*
DI-HiSorb NS	*Direct Immersion HiSorb without salting out*
HS-HiSorb S	*Head space HiSorb with salting out*
HS-HiSorb NS	*Head space HiSorb without salting out*

4.2.3.1 Head-Space Solid Phase Microextraction (HS-SPME)

The WMP solutions (5 mL) were added to a 20 mL amber La-Pha-Pack vial (as described in Section 4.2.2) and equilibrated to 40 ˚C for 10 min, with pulsed agitation of 5 s at 500 rpm using an Agilent GC 80 Autosampler (Agilent Technologies Ireland Ltd., Cork, Ireland). Each sample was pre-incubated at 40 ◦C with pulsed agitation for 10 min. A single SPME 50/30 µm (DVB/CAR/PDMS) fiber (Agilent Technologies Ireland Ltd., Cork, Ireland) was exposed to the headspace above the samples in the vial for 20 min at a depth of 1 cm at 40 ˚C. Following extraction, the SPME fiber was retracted and injected into the gas chromatograph inlet and desorbed for 3 min at 250 ˚C in splitless mode. The fiber was cleaned in a bakeout conditioning station (Agilent Technologies Ireland Ltd., Cork, Ireland), between each sample injection, at 270 ˚C with a nitrogen flow of 6 mL min^{-1} , and blanks were conducted after every triplicate sample to ensure no carryover occurred. A Merlin microseal (Agilent Technologies Ireland Ltd., Cork, Ireland) was used to minimize fiber wear. Each sample was analyzed in triplicate.

4.2.3.2 Thermal Desorption Extraction

A micro-chamber/thermal extractor (Markes International Ltd., Llantrisant, UK) was used for dynamic headspace extraction using industry standard TD tubes packed with Tenax/Carbograph (TEN/CAR) (Markes International Ltd., Llantrisant, UK). The analysis was undertaken in triplicate and the TEN/CAR tubes were preconditioned at 280 ˚C for 1 hr prior to sampling using a TC-20 (Markers International Ltd., Llantrisant, UK). A Unity 2 thermal desorption unit (Markes International Ltd., Llantrisant, UK) was used to concentrate the volatiles and remove excess moisture. A heated transfer line was used to automatically transfer the volatiles

from the Unity 2 to the GC. The WMP solution (5 mL), containing the IS, was added to an inert stainless steel microchamber pot and extracted in the micro-chamber at 40 ˚C at 50 mL min^{-1} in nitrogen for 20 min. Each sorbent tube was desorbed in the Unity 2 thermal desorption unit with a materials emission focusing trap (Markes International Ltd., Llantrisant, UK). The sample tubes were initially pre-purged for 2 min with a 1:20 split, followed by a two-stage desorption. In the first stage, the tubes were ramped to 110 ◦C with a 1:10 split for 10 min, then heated to 280 ˚C for 10 min without a split. The cold trap was set at 30 ˚C, with a trap flow of 50 mL min^{-1}. After tube desorption, a pre-trap fire purge was performed for 2 min, before heating the trap to 300 ˚C at 100 ˚C s^{-1} for 5 min without a split. The transfer line was held at 160 ˚C. Each sample was analyzed in triplicate.

4.2.3.3 Headspace and Direct Immersion Hi-Sorb Extraction

The WMP samples (5 mL) were pipetted into a 20 mL amber La-Pha-Pack vial (Apex Scientific Ltd, Maynooth, Co., Kildare, Ireland) with a HiSorb-P1 inert PDMS probe assembly (Markes International Ltd., Llantrisant, UK) for both HS-HiSorb and for DI-HiSorb. For DI-HiSorb, the HiSorb probe was directly immersed in the liquid WMP sample and sealed. For HS-HiSorb, the probe was placed at a fixed depth of 1 cm above the sample in the vial (care was taken to ensure that the probe remained dry) and sealed. The vials were added to the HiSorb Agitator (Markes International Ltd., Llantrisant, UK) and agitated at 250 rpm for 120 min at 40 ˚C for the DI-HiSorb. The vials were added to the HiSorb agitator at 250 rpm for 180 min at 40 ˚C for the HS-HiSorb. The HiSorb probes were rinsed with deionized water and gently dried with a lint-free tissue prior to insertion into a clean, empty TD tube (Markes International Ltd., Llantrisant, UK), which were end capped using brass long-term storage caps (Markes International Ltd., Llantrisant, UK). The TD tubes were then evaluated in an

identical manner to that described for the TD extraction. Each HiSorb probe was preconditioned at 280 ˚C for 1h between samples using a U-CTE micro-chamber/thermal extractor (Markes International Ltd., Llantrisant, UK).

4.2.4 GC-MS Analysis

The GC-MS system was an Agilent 7890A GC and Agilent 5977B MSD (Agilent Technologies Ireland Ltd., Cork, Ireland). The analysis was undertaken using both a non-polar GC column DB5-MS (60 m × 0.25 mm × 0.25 µm) and a polar GC column HP-Innowax (60 m × 0.25 mm × 0.5 µm) (Agilent Technologies Ireland Ltd., Cork, Ireland). The GC conditions for the non-polar DB5-MS column were as follows: the injector temperature was set at 250 ˚C, while the column was initially at 35 ˚C, then increased to 230 ◦C at 6.5 ˚C min^{-1}, 320 ˚C at 15 ˚C min^{-1}, before being held for 5 min, yielding a total run time of 41 min. The carrier gas helium was held at a constant pressure of 23 psi. The GC conditions for the polar HP-Innowax column were as follows: the injector temperature was set at 250 ˚C, while the column was initially at 40 ˚C for 5 min, then increased to 230 ˚C at 5 ˚C min^{-1}, before being held for 10 min, yielding a total run time of 59 min. The carrier gas helium was held at a constant pressure of 23 psi. The ion source temperature was 220 ˚C and the interface temperature was set at 260 ˚C. The mass spectrometer was in electronic ionization (70 v) mode with the mass range scanned between 35 and 250 amu. The analysis was undertaken using MassHunter Qualitative Analysis software (Agilent Technologies, Palo Alto, CA, USA) with target and qualifier ions and linear retention indices for each compound compared to an in-house library based on mass spectra obtained from NIST 2014 mass spectral library MS searching (v.2.3, Gaithersburg, MD, USA) and an in-house library created using authentic compounds with target and qualifier ions and linear retention indices for each compound using the Kovats index. Spectral

deconvolution was also performed to confirm identification of compounds using the automated mass spectral deconvolution and identification system (AMDIS). Batch processing of the samples was carried out using metaMS (Wehrens et al. 2014), an open source pipeline for GC-MS based untargeted metabolomics. The results for each identified volatile compound were normalized based on the recovery of the 4-methyl-2-pentanol IS for each sample and expressed as a percent of the total volatiles recovered for each sample. Results in all cases were the averages of triplicate analysis.

4.2.5 Data Analysis

Each extraction technique, with or without salting out, were compared using nonpolar and polar GC columns in relation to their ability to extract VOCs in these WMP samples. The results were expressed after normalization in relation to the IS. The sensitivity, selectivity, and reproducibility were compared in terms of: (i) the number of VOCs extracted by each technique, (ii) the percentage of each chemical class extracted by each technique, (iii) the specific identity of each VOC extracted by each technique, (iv) the total abundance of VOCs extracted by each technique (the overall abundance was calculated as the sum of the average abundance of every VOC peak area extracted by that technique, and expressed as a percentage. The extraction technique with the highest total abundance equated to 100% and the others were expressed as a percentage thereof), and (v) the average percentage relative standard deviation of each technique (taken from the relative standard deviation achieved for every VOC recovered in triplicate for each technique) as outlined in (High et al. 2019). Principal component analysis (PCA) biplots of the volatile data were carried out to aid the visual association of volatile compounds using the “factoextra” and “FactoMineR” packages within R (v 3.4.1, R Foundation for Statistical Computing, Vienna, Austria). To visualize the selectivity of each technique in relation to the number of VOCs

recovered, with or without salting out using the non-polar and polar GC columns, Venn diagrams were created with the 4 oval flower model using the Excel template (Microsoft Office, Redmond, WA, USA). Histograms outlining the percent of chemical classes of each extraction technique with or without salting out using the non-polar and polar GC columns were also created using Excel (Microsoft Office, Redmond, WA, USA).

4.3 Results and Discussion

4.3.1 Comparison of Volatile Compounds Extracted from Whole Milk Powder by Each Technique

A summary of all the VOCs identified by each technique, with and without salting out, in terms of percent of abundance, including standard deviations for each VOC, are provided in Table 4.2a (results using a non-polar GC column) and Table 4.2b (results using a polar GC column). In total, the number of individual VOCs identified in these samples across all four extraction techniques, with and without salting out and with both GC column polarities, was 85 (Table 4.3). This is considerably more VOCs than previously found in WMP, which, albeit, only used a single extraction technique (Lloyd et al. 2019; Park and Drake, 2017). Twenty-five VOCs were identified using salting out with SBSE (PDMS) using a non-polar GC column (Park and Drake ,2017) and ten VOCs by HS-SPME (DVB/CAR/PDMS) with salting out using a non-polar GC column (Lloyd et al. 2019). The 85 VOCs identified in this study consisted of 20 ketones, 18 aldehydes, 11 lactones, 11 alcohols, 7 esters, 6 benzene/phenols, 5 furans, 4 terpenes, 2 sulphur compounds, and 1 acid. Most VOCs were identified using the non-polar GC column (80) as opposed to the polar GC column (54) across all extraction techniques, independent of salting out (Table 4.3). A previous study comparing four volatile extraction techniques on natto (a fermented

food) also found considerably more volatiles using a non-polar GC column than a polar GC column; 70 compared to 47 VOCs, with 40 VOCs recovered by both column polarities (Liu et al. 2018). In this study, 30 (ethanol, 1-butanol, 1-octanol, (Z)-4-heptenal, (E)-2-octenal, (E)-2-nonenal, (E)-2-decenal and undecanal, benzeneacetaldehyde, benzyl alcohol, diacetyl, 2-hexanone, 2-octanone, 3-octanone, 2-tridecanone, 2-pentadecanone, 2-heptadecanone, γ-crotonolactone, δ-caprolactone, δ-nonalactone, γ-dodecalactone, δ-undecalactone, δ-tridecalactone, z-dairylactone, longifolene, methyl hexanoate, methyl pyruvate, 2-methyl furan, 2-pentyl furan, and acetic acid) VOCs were extracted, independent of salting out, using the non-polar GC column in comparison to the polar GC column across all four extraction techniques (Table 4.2 a,b). In contrast, only six (1–3-pentanol, 1-nonanol, 2,3- pentanedione, δ-caprolactone, butyl acetate, and 2-ethyl furan) VOCs were extracted across all four extraction techniques, independent of salting out, on the polar GC column, but not on the non-polar GC column (Table 4.2 a,b). Therefore, the VOCs were present in the extract(s) in each case, but did not interact with the particular GC column phase in order to be identified. This further highlights the significance of GC column polarity in volatile extraction/identification by GC-MS.

More VOCs were extracted and identified across all four techniques with salting out (75) and without salting out (72) with the non-polar GC column, than with salting out (48) and without salting (45) on the polar GC column (Table 4.4). Therefore, the impact of salting out was much less than the impact of column polarity in relation to the number of VOCs extracted. In general, salting out modifies the ionic strength of the sample solution with the aim of improving the extraction of polar VOCs, but may adversely impact the extraction of non-polar VOCs (Schiano et al. 2019). However, in practice the impact of salting out in relation to polar and non-polar

VOCs is often unclear as many additional factors relating to the composition of the sample and the parameters of the specific extraction technique may also influence the extraction (Schiano et al. 2019).

Table 4.2 (a) Identification of volatile compounds by each extraction technique with and without salting out using the non-polar GC column. (b) Identification of volatile compounds by each extraction technique with and without salting out using the polar GC column.

(a)

			RI		Identification	DI-HISorb S		DI-HISorb NS		HS-HiSorb S		HS-HiSorb NS		TD S		TD NS		HS-SPME S		HS-SPME NS	
No	Compound	CAS	ORI	REF	Methods	% Area	% Stdev	% Area	% Stdev	% Area	% Stdev	% Area	% Stdev	% Area	% Stdev	% Area	% Stdev	% Area	% Stdev	% Area	% Stdev
										Alcohols											
1	Ethanol	64-17-5	436	426	MS, RI, STD	0.73	0.16	0.27	0.26	0.72	0.31	0.53	0.38	nd	nd	nd	nd	nd	nd	nd	nd
2	1-Butanol	71-36-3	655	675	MS, RI, STD	nd	nd	nd	nd	1.06	0.35	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	1-Pentanol	71-41-0	762	768	MS, RI, STD	0.47	0.22	0.11	0.04	1.3	0.24	1.44	1.03	3.01	0.63	2.07	0.84	2.33	0.43	1.68	0.7
4	1-Hexanol	111-27-3	865	868	MS, RI, STD	0.17	0.07	nd	nd	1.03	0.46	0.72	0.025	1.17	0.37	0.46	0.23	0.87	0.33	0.77	0.3
5	2-Butoxy-ethanol	111-76-2	903	901	MS, RI	0.12	0.04	nd	nd	0.47	0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
6	1-Octen-3-ol	3391-86-4	977	981	MS, RI	0.07	0.04	nd	nd	nd	nd	0.39	0.31	nd	nd	nd	nd	nd	nd	nd	nd
7	2-Ethyl-1-hexanol	104-76-7	1026	1030	MS, RI, STD	0.31	0.14	0.25	0.07	1.96	0.37	nd	nd	8.61	1.67	7.2	1.37	nd	nd	nd	nd
8	1-Octanol	111-87-5	1067	1071	MS, RI, STD	0.22	0.15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
9	α-Terpineol	10482-56-1	1198	1192	MS, RI	nd	nd	nd	nd	nd	nd	0.57	0.28	0.32	0.07	0.26	0.04	nd	nd	nd	nd
										Aldehydes											
10	Acrolein	107-02-8	449	470	MS, RI	0.44	0.22	1.88	0.41	nd	nd	1.26	0.61	nd	nd	nd	nd	nd	nd	nd	nd
11	Butanal	123-72-8	578	596	MS, RI, STD	0.22	0.14	0.09	0.09	nd	nd	nd	nd	nd	nd	nd	nd	5	3.23	nd	nd
12	3-Methyl butanal	590-86-3	647	654	MS, RI, STD	nd	nd	nd	nd	0.61	0.21	0.56	0.19	1.31	0.41	1.33	0.56	4.58	1.41	5.35	2.65
13	Pentanal	110-62-3	697	697	MS, RI, STD	0.9	0.84	0.34	0.11	nd	nd	nd	nd	nd	nd	nd	nd	32.05	12.67	nd	nd
14	Hexanal	66-25-1	799	801	MS, RI, STD	0.94	0.48	0.65	0.26	7.42	4.64	7.45	5.21	6.64	1.66	6.95	2.62	11.43	8.06	16.66	9.82
15	4-Heptenal,(Z)-	6728-31-0	895	902	MS, RI	nd	nd	nd	nd	0.45	0.08	nd	nd	nd	nd	nd	nd	0.32	0.13	nd	nd
16	Heptanal	111-71-7	900	901	MS, RI, STD	0.69	0.15	0.59	0.08	7.11	1.68	7.73	2.42	8.48	1.38	9.38	2.32	5.87	1.93	9.89	2.19
17	Benzaldehyde	100-52-7	967	960	MS, RI, STD	0.15	0.04	0.2	0.05	1.3	0.6	1.86	0.4	1.13	0.37	1.81	1.18	0.34	0.14	1.26	0.12
18	Octanal	124-13-0	1002	1004	MS, RI, STD	0.31	0.08	0.29	0.06	3.21	0.94	3.08	1.09	3.49	0.99	3.78	2.13	0.71	0.12	1.77	0.46
19	Benzeneacetaldehyde	122-78-1	1048	1048	MS, RI, STD	nd	nd	0.04	0.01	nd	nd	1.43	0.64	nd	nd	nd	nd	nd	nd	nd	nd
20	2-Octenal,(E)-	2548-87-0	1059	1057	MS, RI	0.07	0.01	0.1	0.04	nd	nd	nd	nd	0.22	0.09	0.3	0.2	nd	nd	nd	nd
21	Nonanal	124-19-6	1103	1106	MS, RI, STD	1.27	0.39	1.18	0.21	17.52	3.1	16.91	3.47	17.93	2.58	18.26	3.65	1.5	0.24	3.27	0.32
22	2-Nonenal,(E)-	18829-56-6	1160	1160	MS, RI	0.17	0.11	0.11	0.04	0.41	0.13	nd	nd	0.22	0.15	0.2	0.06	nd	nd	nd	nd
23	Decanal	112-31-2	1204	1205	MS, RI, STD	0.36	0.1	0.28	0.1	5.27	1.96	4.57	1.4	1.24	0.46	1.76	0.79	nd	nd	nd	nd
24	2-Decenal,(E)-	3913-81-3	1262	1266	MS, RI	0.12	0.09	0.05	0.01	1.32	0.29	nd	nd	0.11	0.09	0.22	0.21	nd	nd	nd	nd
25	Undecanal	112-44-7	1306	1309	MS, RI, STD	0.07	0.02	0.05	0.02	0.49	0.06	0.48	0.11	0.12	0.03	0.21	0.06	nd	nd	nd	nd
26	2-Undecenal	2463-77-6	1364	1350	MS, RI, STD	0.2	0.09	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
27	Dodecanal	112-54-9	1407	1401	MS, RI, STD	0.12	0.03	0.11	0.03	0.41	0.03	0.53	0.1	0.25	0.07	0.3	0.17	nd	nd	nd	nd
										Benzene/Phenols											
28	Benzene	71-43-2	658	669	MS, RI, STD	0.36	0.17	0.31	0.2	nd	nd	1.33	0.47	6.77	3.33	8.08	5.53	nd	nd	6.2	2.7
29	Toluene	108-88-3	766	763	MS, RI, STD	0.1	0.02	0.09	0.05	0.51	0.24	0.6	0.12	4.54	0.73	3.64	1.08	1.72	0.67	1.6	1.07
30	p-Xylene	106-42-3	870	867	MS, RI, STD	nd	nd	nd	nd	nd	nd	0.77	0.78	3.39	0.6	3.14	0.8	nd	nd	nd	nd
31	o-xylene	95-47-6	898	900	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	1.74	0.75	1.23	0.34	nd	nd	nd	nd
32	Benzyl alcohol	108-95-2	974	995	MS, RI, STD	nd	nd	nd	nd	0.61	0.13	nd	nd	nd	nd	0.23	0.09	nd	nd	nd	nd
33	Phenol	100-51-6	1035	1037	MS, RI, STD	0.16	0.1	0.12	0.04	0.79	0.23	0.76	0.17	0.73	0.31	0.66	0.12	nd	nd	nd	nd
										Ketones											
34	Acetone	67-64-1	451	496	MS, RI, STD	1.62	0.61	nd	nd	8.11	2.62	6.5	1.02	nd	nd	nd	nd	5.4	0.65	nd	nd
35	Diacetyl	431-03-8	548	596	MS, RI, STD	nd	nd	nd	nd	nd	nd	1.75	0.57	3.72	1.67	3.1	0.68	nd	nd	nd	nd
36	Hydroxyacetone	116-09-6	663	657	MS, RI	0.68	0.31	1.03	0.71	0.12	0.11	2.91	2.49	nd	nd	nd	nd	nd	nd	nd	nd
37	2-Pentanone	107-87-9	684	687	MS, RI, STD	nd	nd	0.26	0.08	2.19	0.3	5.38	3.13	4.1	0.53	3.46	0.59	1.21	1.16	5.52	0.91

38	2-Butanone	108-10-1	733	740	MS, RI, STD	nd	nd	nd	nd	1.82	0.39	1.25	0.53	10.04	1.65	7.3	0.83	nd	nd	4.41	1.19
39	Methyl Isobutyl Ketone	108-10-1	735	740	MS, RI, STD	nd	nd	0.09	0.02	0.58	0.11	0.5	0.23	1.48	0.8	0.93	0.23	0.67	0.13	0.76	0.46
40	2-Hexanone	591-78-6	789	790	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.67	0.04	nd	nd
41	2-Heptanone	110-43-0	887	891	MS, RI, STD	1.23	0.28	1.14	0.27	11.95	0.97	10.34	3.18	2.62	0.41	3.85	0.6	13.12	2.71	16.17	6.16
42	2,3-Octanedione	585-25-1	981	967	MS, RI, STD	0.17	0.05	0.16	0.04	1.56	0.37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
43	3-Octanone	106-68-3	982	989	MS, RI	0.05	0.02	0.04	0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
44	2-Octanone	111-13-7	988	992	MS, RI, STD	nd	nd	0.07	0.02	0.39	0.16	0.45	0.07	0.56	0.13	0.55	0.28	nd	nd	nd	nd
45	3,5-Octadien-2-one,(E,E)-	30086-02-3	1069	1072	MS, RI	0.46	0.06	0.37	0.06	1.44	0.38	1.33	0.22	nd	nd	nd	nd	nd	nd	nd	nd
46	Acetophenone	98-86-2	1070	1079	MS, RI, STD	0.09	0.02	nd	nd	0.65	0.13	0.52	0.07	0.68	0.25	nd	nd	nd	nd	nd	nd
47	3,5-Octadien-2-one	38284-27-4	1076	1072	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.69	0.48	0.92	0.23	0.45	0.13
48	2-Nonanone	821-55-6	1088	1094	MS, RI, STD	0.3	0.09	0.51	0.12	3.19	0.19	3.43	0.18	0.54	0.12	0.69	0.05	0.93	0.1	2.33	0.21
49	2-Undecanone	112-12-9	1295	1294	MS, RI, STD	nd	nd	0.08	0.02	nd	nd	0.77	0.08	nd	nd	0.15	0.02	1.64	0.26	nd	nd
50	2-Tridecanone	593-08-8	1494	1480	MS, RI, STD	nd	nd	0.27	0.14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
51	2-Pentadecanone	2345-28-0	1695	1689	MS, RI, STD	0.41	0.4	0.4	0.26	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
52	2-Heptadecanone	2922-51-2	1897	1878	MS, RI, STD	nd	nd	0.54	0.09	0.98	0.39	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
											Lactones										
53	γ-Crotonolactone	497-23-4	912	916	MS, RI	0.21	0.11	0.19	0.11	0.89	0.3	0.92	0.21	nd	nd	nd	nd	nd	nd	nd	nd
54	δ-Caprolactone	823-22-3	1097	1084	MS, RI	nd	nd	0.1	0.03	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
55	δ-Octalactone	698-76-0	1288	1288	MS, RI	nd	nd	0.63	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
56	δ-Nonalactone	3301-94-8	1394	1404	MS, RI	0.22	0.1	0.14	0.03	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
57	δ-Decalactone	705-86-2	1502	1506	MS, RI	31.87	2.9	28.34	4.87	3.81	0.5	3.06	0.45	nd	nd	nd	nd	0.29	0.05	1.05	0.22
58	δ-undecalactone	710-04-3	1602	1627	MS, RI	0.08	0.02	1.68	0.63	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
59	(Z) Dairy lactone	18679-18-0	1664	1675	MS, RI	0.64	0.23	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
60	γ-Dodecalactone	2305-(05)-7	1685	1674	MS, RI	8.56	10.18	13.9	15.58	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
61	δ-Dodecalactone	713-95-1	1717	1719	MS, RI	29.02	3.99	40.05	7.86	1.52	0.25	1.37	0.21	nd	nd	nd	nd	nd	nd	nd	nd
62	δ-Tridecalactone	7370-92-5	1824	1778	MS, RI	0.55	0.18	1.21	0.37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
63	δ-Tetradecalactone	2721-22-4	1930	1938	MS, RI	13.46	1.16	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
											Sulfurous Compounds										
64	Dimethyl sulfide	75-18-3	519	510	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.68	0.65	3.93	1.68
65	Dimethyl disulfide	624-92-0	743	739	MS, RI, STD	0.09	0.02	0.1	0.02	0.19	0.09	nd	nd	1.74	0.32	4.62	1	1.7	0.86	0.85	0.15
											Terpenes										
66	α-Pinene	80-56-8	939	930	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.58	0.47	1.93	1.24
67	3-Carene	13466-78-9	1015	1009	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.49	0.17	1.85	1.02
68	D-Limonene	5989-27-5	1032	1022	MS, RI, STD	0.01	0.02	0.05	0.01	0.33	0.06	0.28	0.06	2.52	2.55	1.18	0.89	0.27	0.04	0.3	0.13
69	Longifolene	475-20-7	1439	1432	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	0.14	0.03	0.17	0.08	nd	nd	nd	nd
											Acids										
70	Acetic acid	64-19-7	535	629	MS, RI, STD	0.21	0.05	0.23	0.14	1.17	1.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
											Esters										
71	Methyl butanoate	623-42-7	716	724	MS, RI, STD	nd	nd	nd	nd	nd	nd	1.05	0.3	0.22	0.04	1.16	0.4	1.5	0.55	2.44	1.14
72	Methyl pyruvate	108-10-1	735	740	MS, RI	0.29	0.19	0.16	0.08	0.54	0.36	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
73	Ethylberzene	100-41-4	859	851	MS, RI, STD	nd	nd	nd	nd	0.53	0.11	nd	nd	0.22	0.15	0.68	0.41	nd	nd	nd	nd
74	Methy hexanoate	106-70-7	918	922	MS, RI	nd	nd	nd	nd	nd	nd	1.31	0.41	nd	nd	nd	nd	0.83	0.1	3.36	1.88
75	Methyl octanoate	111-11-5	1117	1126	MS, RI	nd	nd	nd	nd	nd	nd	0.53	0.43	nd	nd	nd	nd	nd	nd	nd	nd
76	Methyl hexadecanoate	112-39-0	1915	1909	MS, RI	nd	nd	nd	nd	2.16	0.77	2	0.32	nd	nd	nd	nd	nd	nd	nd	nd
											Furans										
77	2-Methyl-furan	534-22-5	602	604	MS, RI, STD	nd	nd	0.17	0.04	0.52	0.42	nd	nd	nd	nd	nd	nd	nd	nd	7.59	4.83
78	2-Pentyl-furan	3777-69-3	989	991	MS, RI, STD	nd	nd	0.05	0.01	0.43	0.19	0.44	0.08	nd	nd	nd	nd	nd	nd	nd	nd
79	2-Furanmethanol	98-0-0	851	850	MS, RI, STD	0.65	0.59	0.59	0.75	0.49	0.35	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
80	Furfural	98-01-1	833	852	MS, RI, STD	0.39	0.19	0.36	0.35	0.51	0.11	0.93	0.24	nd	nd	nd	nd	nd	nd	nd	nd

						(b)															
						Alcohols															
1	α-Terpineol	10482-56-1	1206	1192	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	0.73	0.29	nd	nd	nd	nd	nd	nd
2	1-Pentanol	71-41-0	1262	1250	MS, RI, STD	nd	nd	nd	nd	2.34	1.86	2.17	3.35	nd	nd	nd	nd	nd	nd	nd	nd
3	1-Hexanol	111-27-3	1365	1355	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	1.11	0.4	0.88	0.38	1.44	0.86	0.87	0.91
4	1-Penten-3-ol	111-27-3	1365	1355	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	1.89	1.02	1.25	0.95	2.06	1.76	nd	nd
5	2-Butoxy-ethanol	111-76-2	1423	1405	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.45	1.4	nd	nd	nd	nd
6	1-Octen-3-ol	3391-86-4	1460	1450	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.67	0.1	nd	nd	nd	nd
7	2-Ethyl-1-hexanol	71-41-0	1502	1491	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	12.02	1.89	15.69	3.3	4.18	0.51	2.18	0.81
8	1-Nonanol	143-08-8	1673	1660	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.81	0.33	nd	nd	nd	nd
9	Acrolein	107-02-8	449	470	MS, RI	0.2	0.24	0.31	0.05	4.55	2.02	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	Butanal	123-72-8	578	596	MS, RI, STD	nd	nd	0.12	0.03	nd	nd	nd	nd	1.04	0.08	1.3	0.24	nd	nd	nd	nd
11	3-Methyl-butanal	590-86-3	652	654	MS, RI, STD	0.28	0.05	nd	nd	nd	nd	nd	nd	0.59	0.48	1.08	0.65	2.2	1.13	17.4	17.85
12	Pentanal	110-62-3	994	979	MS, RI, STD	0.68	0.26	0.52	0.23	1.68	1.05	2.22	1.93	7.38	2.74	nd	nd	11.23	5.97	nd	nd
13	Hexanal	66-25-1	1098	1083	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	2.6	0.64	3.87	0.58	5.9	0.33	4.55	6.02
14	Heptanal	111-71-7	1204	1184	MS, RI, STD	0.57	0.1	0.4	0.13	9.62	4.17	13.91	6.8	4.01	0.92	4.81	0.89	10.47	3.64	24.2	10.48
15	Octanal	124-13-0	1310	1289	MS, RI, STD	nd	nd	0.4	0.06	2.34	0.43	4.32	0.98	1.85	0.78	2.43	1.09	nd	nd	nd	nd
16	Nonanal	124-19-6	1416	1391	MS, RI, STD	1.02	0.24	0.89	0.31	1.27	0.96	8.41	6.43	10.12	3.05	12.09	2.97	0.62	0.37	nd	nd
17	Decanal	112-31-2	1523	1498	MS, RI, STD	0.48	0.12	0.61	0.31	16.88	9.22	8.54	12.98	1.44	0.24	2.99	1.03	nd	nd	nd	nd
18	Benzaldehyde	100-52-7	1570	1520	MS, RI, STD	0.52	0.1	0.33	0.07	5.63	4.25	4.56	4.43	1.21	0.31	2.55	0.45	0.9	0.11	2.28	1.06
19	Dodecanal	112-54-9	1733	1710	MS, RI, STD	nd	nd	nd	nd	0.31	0.47	6.08	1.32	nd	nd	nd	nd	nd	nd	nd	nd
						Benzene/Phenols															
20	Toluene	108-88-3	766	763	MS, RI, STD	1.1	0.17	nd	nd	nd	nd	nd	nd	0.56	0.16	0.75	0.2	0.53	0.41	2.62	0.96
21	Benzene	71-43-2	955	957	MS, RI, STD	0.34	0.2	0.26	0.17	nd	nd	nd	nd	1.49	0.37	2	0.72	nd	nd	nd	nd
22	p-Xylene	106-42-3	1155	1138	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.86	0.17	nd	nd	nd	nd
23	o-Xylene	95-47-6	1156	1186	MS, RI, STD	0.35	0.04	nd	nd	nd	nd	nd	nd	2.02	1.21	nd	nd	nd	nd	nd	nd
24	Phenol	108-95-2	2042	2039	MS, RI, STD	nd	nd	nd	nd	1.14	1.37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
						Ketones															
25	Hydroxyacetone	116-09-6	663	657	MS, RI	2.68	1.68	2.49	1.14	nd	nd	0.51	0.79	nd	nd	nd	nd	nd	nd	nd	nd
26	2-Pentanone	107-87-9	684	687	MS, RI, STD	0.74	0.43	nd	nd	nd	nd	nd	nd	5.18	3.58	nd	nd	nd	nd	8.65	2.87
27	Methyl isopropyl ketone	108-10-1	735	740	MS, RI, STD	nd	nd	0.45	0.18	nd	nd	nd	nd	nd	nd	nd	nd	8.97	1.96	nd	nd
28	Acetone	67-64-1	825	819	MS, RI, STD	1.6	0.62	1.17	0.33	2.89	1.09	3.46	1.67	26.04	7.92	29.76	8.27	9.61	2.67	6.07	5.05
29	2-Heptanone	110-43-0	887	891	MS, RI, STD	3.04	1.15	0.36	0.1	1.44	0.51	1.84	0.79	6.33	1.89	nd	nd	17.94	4.91	nd	nd
30	2-Butanone	108-10-1	913	907	MS, RI, STD	0.28	0.17	nd	nd	nd	nd	nd	nd	6.88	2.11	8.38	2.44	5.97	4.05	5.39	1.2
31	Acetophenone	98-86-2	1070	1079	MS, RI, STD	0.43	0.07	nd	nd	nd	nd	nd	nd	0.7	0.22	nd	nd	nd	nd	nd	nd
32	2,3-Pentanedione	600-14-6	1073	1058	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	0.82	0.34	0.95	0.33	nd	nd	nd	nd
33	2-Nonanone	821-55-6	1410	1390	MS, RI, STD	1.13	0.56	0.98	0.23	nd	nd	nd	nd	0.95	0.11	1.8	0.26	2.53	0.62	3.63	1.59
34	3,5-Octadien-2-one	38284-27-4	1549	1522	MS, RI	nd	nd	nd	nd	11.26	11.15	7.02	5.4	0.95	0.67	0.83	0.28	nd	nd	2.29	0.94
35	3,5-Octadien-2-one,(E,E)-	30086-02-3	1551	1570	MS, RI	1.38	0.83	1	0.47	2.84	2.36	0.66	1.32	nd	nd	nd	nd	0.92	0.74	nd	nd
36	2-Undecanone	112-12-9	1622	1598	MS, RI, STD	nd	nd	0.68	0.17	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
						Lactones															
37	δ-Caprolactone	823-22-3	1864	1791	MS, RI	0.25	0.24	0.33	0.09	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
38	δ-Octalactone	698-76-0	2037	1976	MS, RI	nd	nd	nd	nd	1.53	2.29	nd	nd	nd	nd	nd	nd	4.21	0.26	nd	nd
39	δ-Decalactone	705-86-2	2242	2190	MS, RI	nd	nd	nd	nd	12.46	3.67	14.39	3.58	nd	nd	nd	nd	nd	nd	nd	nd
40	δ-Dodecalactone	713-95-1	2570	2436	MS, RI	62.94	1.99	62.33	1.64	5.56	4.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
41	δ-Tetradecalactone	7370-92-5	2892	2688	MS, RI	19.61	1.57	24.59	2.66	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

					Sulfurous Compounds														
42	Dimethyl disulfide	624-92-0	743	739	MS, RI, STD	0.38	0.04	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
43	Dimethyl sulfide	75-18-3	755	754	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	0.36	0.45	0.69	0.34	4.24	2	3.72
					Terpenes														
44	α-Pinene	80-56-8	1035	1028	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.9	0.22	2.09
45	3-Carene	13466-78-9	1152	1147	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.64	0.19	1.45
46	D-Limonene	5989-27-5	1218	1200	MS, RI, STD	nd	nd	0.16	0.07	nd	nd	nd	nd	0.8	0.41	1.1	0.27	nd	nd	7.72
					Esters														
47	Methyl butanoate	623-42-7	1001	982	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.46	0.87	4.9
48	Butyl acetate	123-86-4	1087	1074	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	0.93	0.43	nd	nd	nd	nd	nd
49	Ethylbenzene	100-41-4	1120	1129	MS, RI, STD	0.06	0.01	nd	nd	nd	nd	nd	nd	0.66	0.06	0.8	0.11	nd	nd	nd
50	Methyl hexadecanoate	112-39-0	1915	1909	MS, RI	nd	nd	nd	nd	3.67	1.28	1.13	1.92	nd	nd	nd	nd	nd	nd	nd
51	Methyl octanoate	111-11-5	1407	1385	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.05	0.07	nd
					Furans														
52	2-Ethyl-furan	3208-16-0	968	950	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.01	0.41	nd
53	Furfural	98-01-1	1497	1461	MS, RI, STD	nd	nd	0.8	0.36	8.91	7.71	17.33	10.71	nd	nd	nd	nd	nd	nd	nd
54	2-Furanmethanol	98-00-0	1681	1660	MS, RI, STD	nd	nd	0.81	0.73	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Results are expressed as relative abundance normalized to internal standard (% area, % relative standard deviation). CAS (Chemical Abstracts Service number). MS (identity confirmed by mass spectra to an in-house library). RI (linear retention index as determined). ORI (linear retention index as determined in this study). REF (relevant linear retention index as published reference, if available). STD (an internal standard was used to confirm identification). nd (not determined).

Table 4.3 The numbers of volatile organic compounds extracted in whole milk powder samples.

No of VOCs Extracted	Non-polar GC Column	Polar GC Column
With salting out	75	48
Without Salting out	72	45
Total	80	54
Overall Total	85	

Table 4.4 Numbers, abundance, and reproducibility of volatile organic compounds in whole milk powder samples extracted by each technique with and without salting out and for polar and non-polar GC columns.

NON-POLAR GC COLUMN								
Extraction Technique	**HS-SPME S**	**TD S**	**Di-HiSorb S**	**HS-HiSorb S**	**HS-SPME NS**	**TD NS**	**Di-HiSorb NS**	**HS-HiSorb NS**
No of VOCs	28	34	49	46	25	36	51	42
Abundance %	1.7	1.3	34.2	7.5	3.9	2.2	100	11.6
Average RSD %	39.3	33.7	45.3	33.5	38.3	38.1	40.5	35.2
POLAR GC COLUMN								
Extraction Technique	**HS-SPME S**	**TD S**	**Di-HiSorb S**	**HS-HiSorb S**	**HS-SPME NS**	**TD NS**	**Di-HiSorb NS**	**HS-HiSorb NS**
No of VOCs	23	28	23	19	17	25	22	16
Abundance %	1.2	2.5	39.7	4	1.3	3.1	100	7.7
Average RSD %	37.4	39.1	37.6	71.1	63.8	32.5	34.6	90

No. of VOCs (number of volatile organic compounds). Abundance %, (the greatest abundance achieved by a single extraction technique equated to 100% and the remaining extraction techniques were expressed as a percentage thereof). Average RSD % (the average percentage relative standard deviation of all VOCs for extraction technique).

4.3.2 The Percentage of Each Chemical Class Extracted from Whole Milk Powder by Each Technique

Figure 4.1 a is histogram highlighting the breakdown of the percentage of each chemical class extracted by each of the four techniques using the non-polar GC column with and without salting out. Figure 1b is the corresponding figure for the polar GC column. It is immediately apparent that significant differences existed in relation to the type and percentage of each chemical class extracted by each technique, influenced by GC column polarity and, to a lesser extent, salting out. All DI-HiSorb techniques (DI-HiSorb S and DI HiSorb NS), independent of GC column polarity, were characterized by the large volume of lactones extracted (>82%), which differs considerably to all of the other extraction techniques. A similar result was found for SBSE, which is a comparable technique to DI HiSorb that also used PDMS as the sorbent phase (High et al. 2019). The only other significant number of chemical classes extracted by DI-HiSorb NS were aldehydes, ketones, and furans, but DI-HiSorb NS did not extract any alcohols, sulphur compounds, acids, terpenes, or esters with the non-polar GC column (but did extract low levels, from 0.5–0.63%, with the polar GC column). The DI-HiSorb S slightly modified the percentage recovery of some chemical classes in comparison to the DI-HiSorb NS. The percentages of chemical classes extracted by DI-HiSorb (DI-HiSorb S and DI-HiSorb NS) using the non-polar GC column were similar independent of salting out (although slightly more alcohols were extracted with salting out). The percentage of each chemical class extracted by HS-HiSorb (HS-HiSorb S and HS-HiSorb NS) for each GC column polarity differed considerably to that attained by DI-HiSorb. This same trend was also apparent when comparing HSSE (similar to HS-HiSorb) and SBSE (similar to DI-HiSorb) (Strobl et al. 2007). HS-HiSorb S and HS-HiSorb NS attained a much lower percentage of lactones on the polar GC column (~14–20%) and with the non-polar GC column (~5–

6%) than DI-HiSorb S and DI-HiSorb NS. HS-HiSorb S and HS-HiSorb NS had a much higher percentage recovery of aldehydes (~42–48%) than DI-HiSorb S and DI-HiSorb NS across both GC column polarities. The percentage of ketones extracted by HS-HiSorb S and HS-HiSorb NS varied considerably depending upon GC column polarity with levels at ~32–35% with the non-polar GC column, and ~14–19% with the polar GC column. The greatest difference in the percentage recovery of chemical classes between HS-HiSorb S and HS-HiSorb NS in relation to GC column polarity, apart from lactones, was for the recovery of furans, with only 1–2% recovered using the non-polar GC column, but 9–17% recovered on the polar GC column. The percentage recovery of esters by HS-HiSorb S and HS-HiSorb NS were relatively high in comparison to the other extraction methods at ~3–5% for the non-polar GC column and ~2–4% for the polar GC column. HS-HiSorb NS did not recover any terpenes, sulphur compounds, acids, or benzene/phenol compounds using the polar GC column, nor sulphur compounds or acids using the non-polar GC column. TD S and TD NS were characterized as having a high percentage recovery of aldehydes (~30–45%), ketones (~21–48%), and alcohols (~10–22%) that varied with both GC column polarity and salting out. TD, independent of salting out, recovered the highest percentage of benzene/phenol compounds using the non-polar GC column in comparison to all the other extraction techniques. TD S or TD NS did not extract any lactones, acids, or furans independent of salting out or GC column polarity. The impact of salting out was minimal in relation to TD, however the percentage of alcohols decreased with salting out using the polar GC column, but increased using the non-polar GC column. Overall, the combination of TEN/CAR should enable a wide range of VOCs to be recovered, as TEN is particularly suited to the extraction of non-polar and slightly polar VOCs, apart from very low molecular weight(<C6) VOCs, which

CAR can extract (Schieweck et al. 2018).The percentage recovery of many chemical classes differed most in relation to both GC column polarity and salting out for HS-SPME than any of the other extraction techniques. Overall, HS-SPME recovered all chemical classes except for acids, independent of salting out and GC column polarity, or furans and lactones by HS-SPME NS using the polar GC column, or furans by HS-SPME S using the non-polar GC column. Overall HS-SPME was characterized by a high percentage recovery of aldehydes (~31–62%), which was reduced with the inclusion of salting out independent of both GC column polarities. For HS-SPME S, the percentage recovery of ketones (25–46%) was much higher using the polar GC column. HS-SPME NS also recovered many more terpenes independent of GC column polarity. Previous studies noted that the DVB/CAR/PDMS multiphase SPME fibers tend to extract the most volatile low boiling point VOCs more effectively (Salum et al. 2017), which corresponds with the results of this study.

No acids were recovered by any technique using the polar GC-column, however it worth pointing out that only one acid (acetic acid) was identified in these WMP samples.

4.3.3 The Relationship between the Individual Volatile Compound Chemical Classes Extracted by Each Technique in the Whole Milk Powder

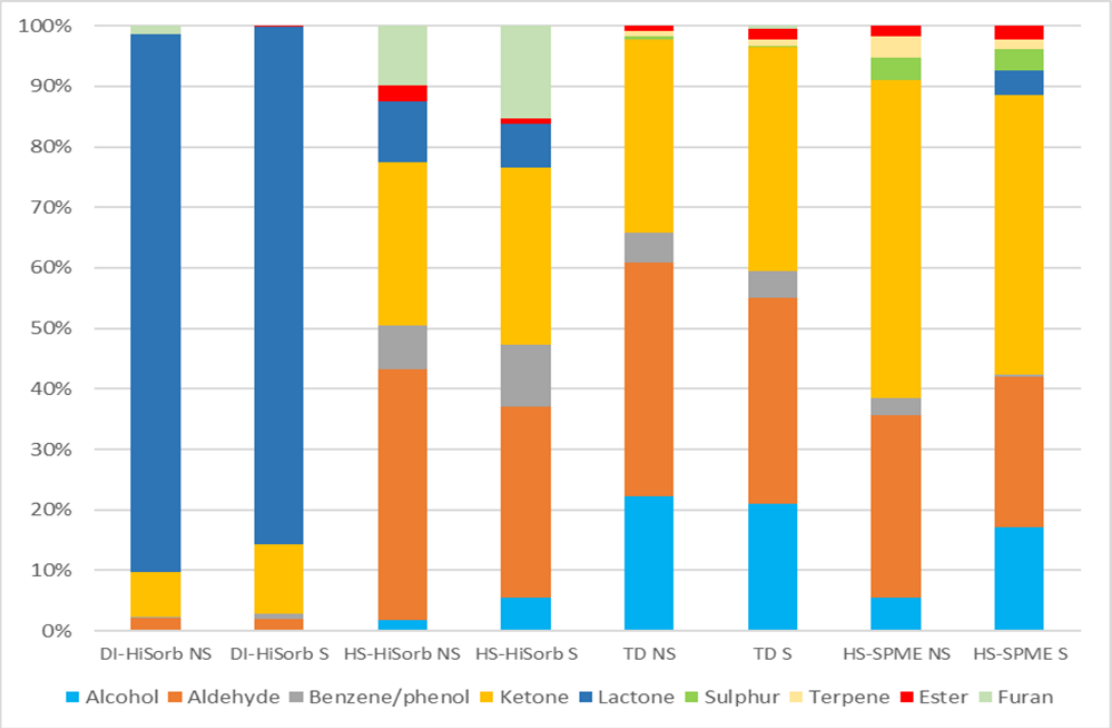
Figure 4.2a,b are principal component analysis (PCA) plots, highlighting the associations of the different extraction techniques with each chemical class. The chemical class data used to generate the PCA was based on the percentage of each chemical class, rather than individual VOCs determined for each technique (with and without salting out for each GC column polarity), to visualize the associations between chemical class and individual extraction techniques, rather than individual VOC. Figure 4.2a relates to each chemical class with and without salting out using the non-

polar GC column. The total level of discrimination was 44.8% (PCA 1 23.6% and PCA 2 20.6%). It is immediately apparent that salting out did not have a major impact on the individual extraction techniques, as with and without salting out (S and NS) for each technique are very closely associated with each other. Both DI-HiSorb S and DI-HiSorb NS were most strongly associated with lactones as previously mentioned, and were separate from HS-HiSorb S and HS-HiSorb NS, which were not strongly associated with any chemical class, but more so with esters and ketones. Although both HS-HiSorb techniques appeared closely associated with the chemical group acids, this was more a reflection of using the overall percentage of each chemical class data, the fact that only one VOC (acetic acid) made up this chemical class, and due to the relationship of this acid with the other extraction techniques. In fact, HS-HiSorb NS did not extract acetic acid. TD (TD S and TD NS) were most strongly associated with aldehydes, benzene/phenols, alcohols, and ketones, while HS-SPME (HS-SPME S and HS-SPME NS) were most closely associated with terpenes and sulphur compounds. Although the HS-SPME techniques did extract a high percentage of ketones and aldehydes, the association was less obvious as the relationship of these chemical classes to the other extraction techniques also influenced their position on the PCA (as stated earlier, the overall percentage of each chemical class data was used to generate the PCA rather than individual VOC.

Figure 4.2b highlights the same associations of chemical classes with each extraction technique with and without salting out, but using the polar GC column. The level of overall discrimination was less, at 39.7% (PCA 1 22.2% and PCA 2 17.5%), than that achieved in Figure 4.2a. As acetic acid was found using the polar GC column, the acid chemical class is not present. Some similar patterns are evident with the polar GC column as found with the non-polar GC column. It is also evident that the impact

of salting out was minimal due to the close association of each individual technique with and without salting out (S and NS). For the polar GC column, both DI-HiSorb (DI-HiSorb S and DI-HiSorb NS) and HS-HiSorb (HS-HiSorb S and HS-HiSorb NS) were much more closely associated with lactones and furans. However, there were some anomalies in that DI-HiSorb, independent of salting out, had little or no recovery of furans, and that both DI-HiSorb and HS-HiSorb recovered significant levels of ketones, which were not reflected in the PCA. As previously mentioned, this is due to the use of the percentage of chemical class data rather than individual VOC data to create the PCA. HS-SPME (HS-SPME S and HS-SPME NS) were most closely associated with sulphur compounds, terpenes, and esters, but also with aldehydes and ketones. TD (TD S and TD NS) were most closely associated with alcohols and benzene/phenol VOCs, and, to a lesser extent, with aldehydes and ketones.

(A)


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
DI-HiSorb NS
DI-HiSorb S
HS-HiSorb NS
HS-HiSorb S
TD NS
TD S
HS-SPME NS
HS-SPME S
Alcohol
Aldehyde
Benzene/phenol
Ketone
Lactone
Sulphur
Terpene
Ester
Furan

(B)

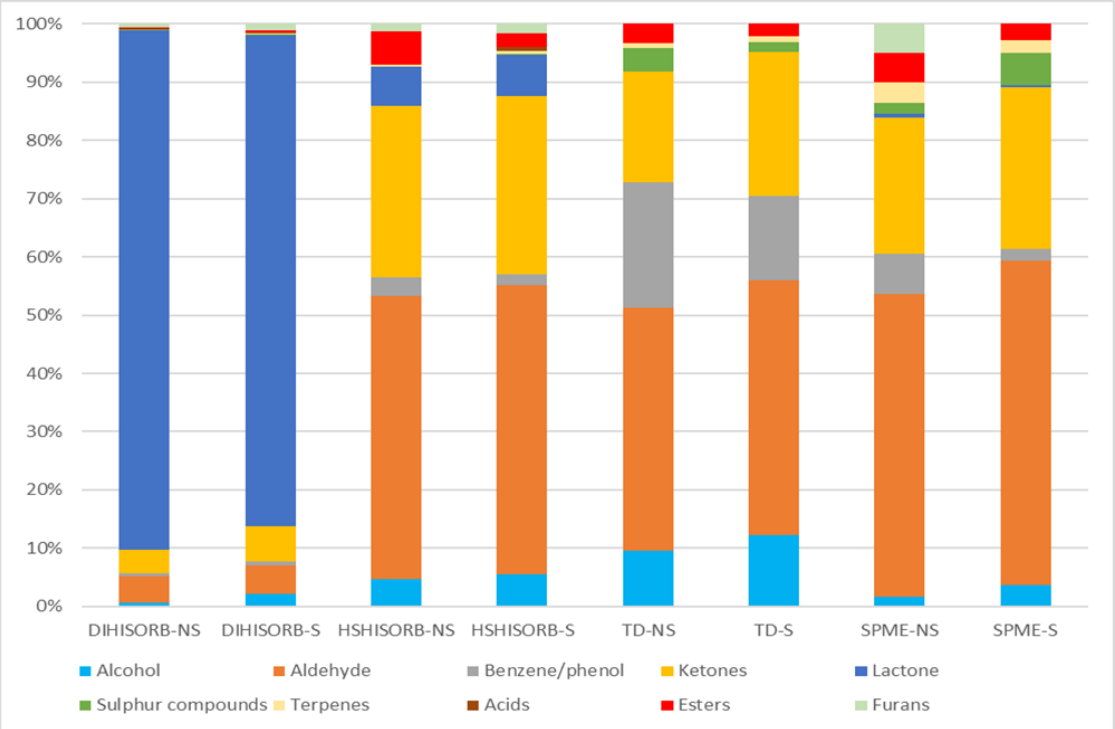
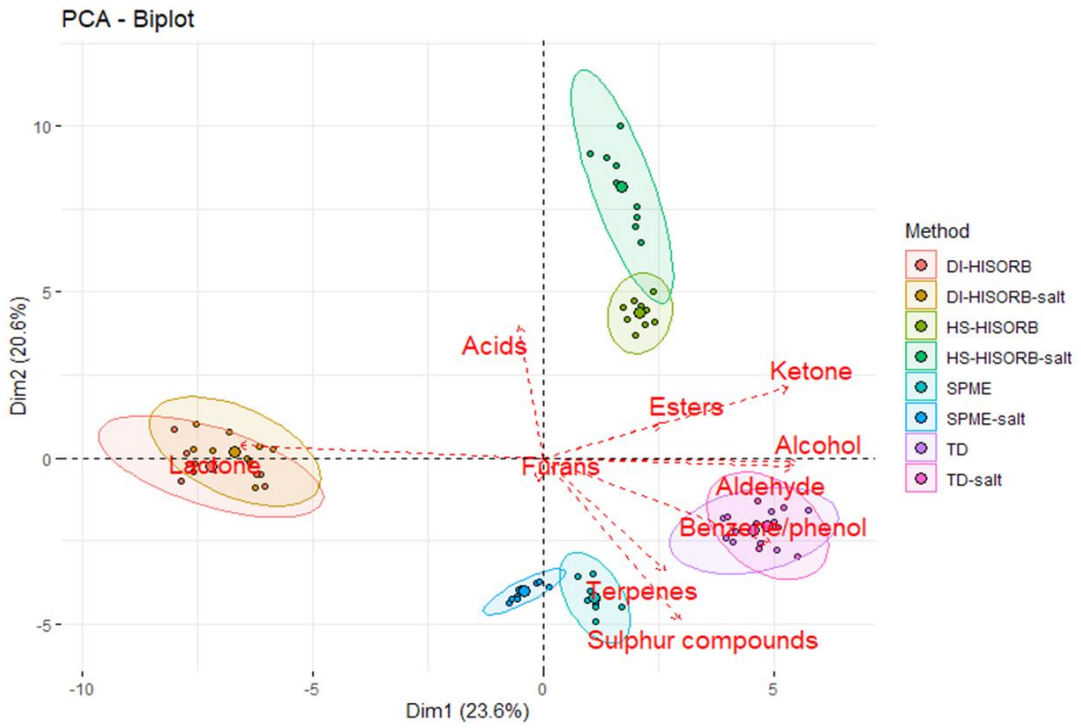

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
DIHISORB-NS
DIHISORB-S
HSHISORB-NS
HSHISORB-S
TD-NS
TD-S
SPME-NS
SPME-S
Alcohol
Aldehyde
Benzene/phenol
Ketones
Lactone
Sulphur compounds
Terpenes
Acids
Esters
Furans

Figure 4.1 (a) The percentage of each chemical class extracted by the four extraction techniques using the non-polar GC column with and without salting out, and (b) the percentage of each chemical class extracted by the four extraction techniques using the polar GC column with and without salting out. Direct Immersion HiSorb without salting out (DIHiSorb NS), Direct Immersion HiSorb with salting out (DI-HiSorb S), Headspace HiSorb without salting out (HS-HiSorb NS), Headspace HiSorb with salting out (HS-HiSorb S), Thermal Desorption without salting out (TD-NS), Thermal Desorption with salting out (TD S), Headspace Solid Phase Microextraction without salting out (HS-SPME NS), Headspace Solid Phase Microextraction with salting out (HS-SPME S).

(A)


PCA - Biplot
Dim2 (20.6%)
Dim1 (23.6%)
Acids
Ketone
Esters
Alcohol
Furans
Lactone
Aldehyde
Benzene/phenol
Terpenes
Sulphur compounds
Method
DI-HISORB
DI-HISORB-salt
HS-HISORB
HS-HISORB-salt
SPME
SPME-salt
TD
TD-salt

(B)

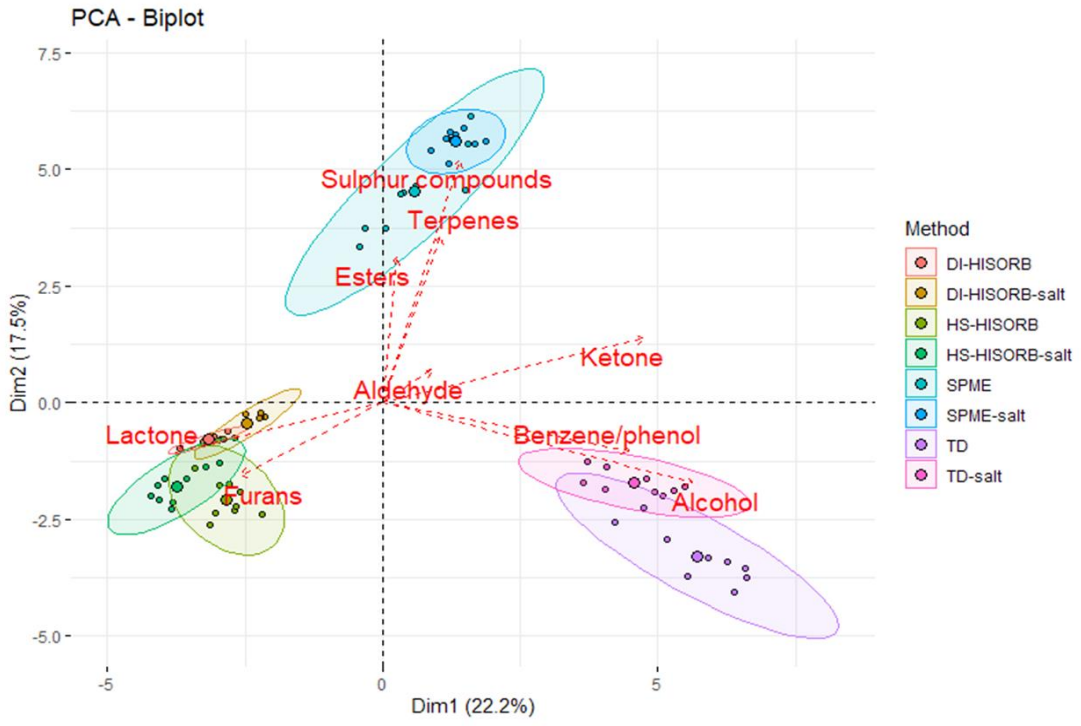

PCA - Biplot
Dim2 (17.5%)
Dim1 (22.2%)
Sulphur compounds
Terpenes
Esters
Ketone
Aldehyde
Lactone
Benzene/phenol
Furans
Alcohol
Method
DI-HISORB
DI-HISORB-salt
HS-HISORB
HS-HISORB-salt
SPME
SPME-salt
TD
TD-salt

Figure 4.2 (a) Principal component analysis of the volatile organic compounds (as per chemical class) per individual extraction technique, with and without salting out, using the non-polar GC column, and (b) principal component analysis of the volatile organic compounds (as per chemical class) per individual extraction technique, with and without salting out, using the polar GC column. Direct Immersion HiSorb without salting out (DI-HiSorb NS), Direct Immersion HiSorb with salting out (DI-HiSorb S), Headspace HiSorb without salting out (HS-HiSorb NS), Headspace HiSorb with salting out (HS-HiSorb S), Thermal Desorption without salting out (TD-NS), Thermal Desorption with salting out (TD S), Headspace Solid Phase Microextraction without salting out (HS-SPME NS), Headspace Solid Phase Microextraction with salting out (HS-SPME S).

4.3.4 The Selectivity of Each Extraction Technique

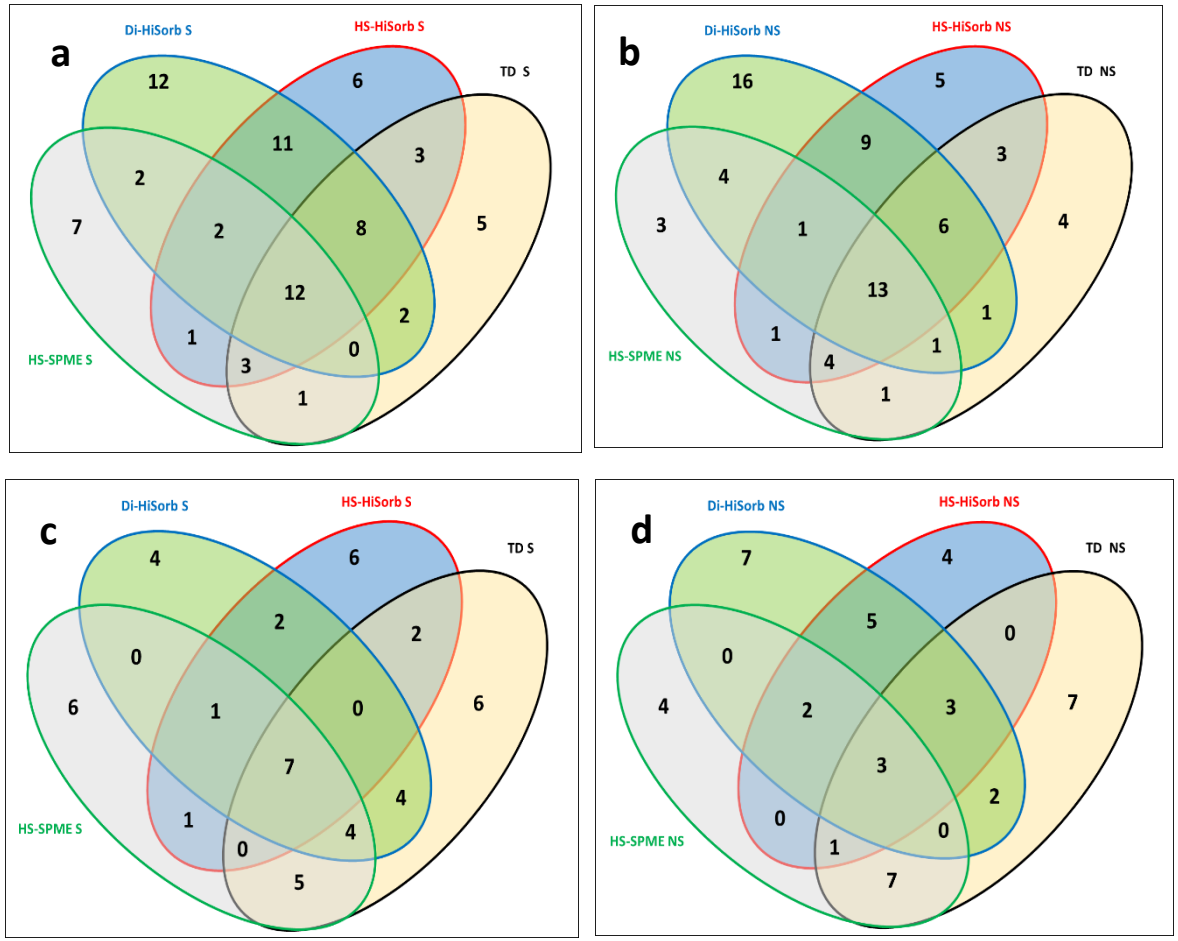
Figure 4.3 is a series of Venn diagrams representing the selectivity of each technique in relation to (a) with salting out using the non-polar GC column, (b) without salting out using the non-polar GC column, (c) with salting out using the polar GC column, and (d) without salting out using the polar GC column. These figures highlight commonalities in relation to VOCs extracted by each technique and those recovered only by each individual extraction technique. It is immediately apparent that more VOCs were extracted using the non-polar GC columns (Figure 4.3 a,b) than with the polar GC column (Figure 4.3 c,d), as previously stated. However, these figures give a better insight into the discrepancies and commonalities with regard to the numbers of VOCs extracted across all four techniques. The greatest number of VOCs were associated with the two HiSorb techniques (DI-HiSorb and HS-HiSorb).

More commonalities were evident in relation to specific VOCs between both Hi-Sorb techniques, which is not surprising as they both utilize the same sorbent phase (PDMS), despite the fact that, in general, PDMS is regarded as less useful for the recovery of polar VOCs (Prieto et al. 2015). Thus overall, Hi-Sorb was quite effective for the general recovery of VOCs in WMP. Considerably fewer synergies were evident between both Hi-Sorb techniques and HS-SPME and TD. As mentioned, PDMS is thought to be more effective for the recovery of less polar VOCs than HS-SPME with multiple fiber phases (Schiano et al. 2019). HS-SPME appeared to be very effective at recovering terpenes and sulphur compounds independent of column polarity and salting out. While quite poor at recovering lactones, furan, and acids, this was also dependent upon GC column polarity and, to a much lesser extent, salting out. The DVB/CAR/PDMS multiphase SPME fibers tended to extract very volatile low boiling point VOCs more effectively (Salum et al. 2017). In addition, as DVB is a polar porous

coating, it is quite efficient in extracting polar compounds and, thus, was useful for sulphur VOCs (High et al. 2019; Salum et al. 2017; Merkle et al. 2015), as evident in this study. The TEN components of the TD phase were less useful for very volatile VOCs, but compensated to some extent by the inclusion of CAR in the packing material (Schieweck et al. 2018).

In summary, 12 VOCs were extracted by every technique (1-pentanol, 1-hexanol, hexanal, heptanal, octanal, nonanal, benzaldehyde, toluene, 2-heptanone, 2-nonanone, dimethyl sulfide, and D-limonene) (Table 4.2a) with salting out using the non-polar GC column (Figure 4.3a). Thirteen 13 VOCs (1-pentanol, hexanal, heptanal, octanal, nonanal, benzaldehyde, toluene, benzene, 2-pentanone, 2-heptanone, methyl isopropyl ketone, 2-nonanone, and D-limonene) (Table 4.2a) were extracted by every technique without salting out using the non-polar GC column (Figure 4.3b). Whereas, in relation to the polar GC column, only seven VOCs (pentanal, heptanal, nonanal, benzaldehyde, decanal, acetone, and 2-heptanone) (Table 4.2b) were extracted with salting out (Figure 4.3c), and only three VOCs (heptanal, benzaldehyde, and acetone) (Table 4.2b) without salting out (Figure 4.3d).

The only VOCs extracted by all four extraction techniques independent of GC column polarity and salting out were heptanal and benzaldehyde (Table 4.2a,b). This likely reflects a combination of their relative abundance and chemical properties, which enabled them to be more easily recovered by each technique, despite the range of different phases and GC column polarities.


a
Di-HiSorb S
HS-HiSorb S
TD S
HS-SPME S
12
6
11
3
2
7
2
8
5
12
2
1
0
3
1
b
Di-HiSorb NS
HS-HiSorb NS
TD NS
HS-SPME NS
16
5
9
3
4
3
1
6
4
13
1
1
1
4
1
c
Di-HiSorb S
HS-HiSorb S
TD S
HS-SPME S
4
6
2
2
0
6
1
0
6
7
4
1
4
0
5
d
Di-HiSorb NS
HS-HiSorb NS
TD NS
HS-SPME NS
7
4
5
0
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4
2
3
7
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2
0
0
1
7

Figure 4.3 Venn diagrams of the number of volatile organic compounds extracted by each technique, including commonalities (a) with salting out using the non-polar GC column, (b) without salting out using the non-polar GC column, (c) with salting out using the polar GC column, and (d) without salting out using the polar GC column. Direct Immersion HiSorb without salting out (DI-HiSorb NS), Direct Immersion HiSorb with salting out (DI-HiSorb S), Headspace HiSorb without salting out (HS-HiSorb NS), Headspace HiSorb with salting out (HS-HiSorb S), Thermal Desorption without salting out (TD-NS), Thermal Desorption with salting out (TD S), Headspace Solid Phase Microextraction without salting out (HS-SPME NS), Headspace Solid Phase Microextraction with salting out (HS-SPME S).

4.3.5 The Abundance of Volatile Organic Compounds in Whole Milk Powder by Each Extraction Technique

The greatest abundance of VOCs extracted across all techniques was achieved by DI-HiSorb NS, independent of GC column polarity (Table 4). As mentioned, all other abundances were expressed as a percentage of the technique with the greatest abundance (i.e., DI-HiSorb NS equated to 100% abundance). The abundance of VOCs recovered by DI-HiSorb NS was also impacted by GC column polarity, as abundances achieved by the non-polar GC column were ~41% lower than that achieved by the polar GC column (data not shown). Therefore, even though more VOCs were recovered using the non-polar GC column by DI-HiSorb NS, the total abundances were lower. The average total abundance for DI-HiSorb S, HS-HiSorb NS, HS-HiSorb S, HS-SPME NS, TD NS, HS-SPME S and TD S for the non-polar GC column was 34.2%, 11.6%, 7.5%, 3.9%, 2.2%, 1.7%, and 1.3%, respectively. A similar trend was evident for the polar GC column, where DI-HiSorb S, HS-HiSorb NS, HS-HiSorb S, TD NS, TD S, HS-SPME NS, and HS-SPME S were 39.7%, 7.7%, 4.0%, 3.1%, 2.5%, 1.3%, and 1.2%, respectively, of that attained by DI-HiSorb NS (100%). The much greater abundance of the DI-HiSorb technique appears mainly due to the advantages of DI over HS, namely, the high capacity of the phase (which is much greater than SPME), and the selectivity of the phases' ability to extract lactones (more volume and quantities of lactones). The abundance of DI-HiSorb was impacted by salting out, as the addition of salt decreased abundances by approximately two thirds. The abundances of TD and HS-SPME were similar and slightly less than those achieved for HS-HiSorb. Therefore, the dynamic nature of TD, in comparison to the static HS-SPME and HS-HiSorb techniques, did not significantly impact VOC abundance. Differences in capacity and selectivity of the difference phases had a lesser impact than DI versus HS on abundance. A study comparing the SBSE (similar to DI-HiSorb),

HSSE (similar to HS-HiSorb), and HS-SPME also found a similar trend for the extraction of fruit VOCs (Barba et al. 2017), in that SBSE extracted more and a greater abundance of VOCs than HSSE and HS-SPME.

4.3.6 The Reproducibility of Each Extraction Technique

The reproducibility of each technique was assessed by comparing the average percent relative standard deviation, (average of the percent relative standard deviation of every VOC for each technique) (Table 4.4) for each extraction technique in relation to column polarity and salting out. In terms of the non-polar GC column with and without salting out, the average standard deviation varied from 33.5% (HS-HiSorb S) to 45.3% (DI-HiSorb S). The average standard deviation range was greater for the polar GC column with or without salting out, from 32.5% for TD NS to 90.0% for HS-HiSorb NS. Overall reproducibility was lower for the HS techniques (HS-HiSorb S, HS-SPME NS, and HS-HiSorb NS) for the polar GC column than any of the other techniques. A recent study on spray dried sheep milk found that the average reproducibility (again, based on average relative standard deviation) of HS-SPME and SBSE was better than HSSE using a non-polar column (High et al. 2019). It must be stated that the average percent relative standard deviation is a relatively crude approach to assess reproducibility. Nevertheless, it was used in this study for comparative convenience across the four techniques due to the number of factors assessed and the significant number of VOCs extracted. The average percentage relative standard deviation does not account for differences in the numbers, abundances, or the selectivity of each technique (impacted by the chemical properties of VOCs and the phases used in each extraction technique) all of which can have an impact on reproducibility. Thus, the individual relative percentage standard deviation values attained for each VOC across each technique with or without salting out for

each GC column polarity provided a more in-depth, true reflection of reproducibility (Table 4.2 a,b).

4.4 Conclusions

The evaluation of WMP by these four extraction techniques has highlighted the extent of VOCs present, which consisted mainly of ketones, aldehydes, lactones, and alcohols with lower numbers of esters, benzenes, phenols, furans, terpenes, sulphur compounds, and one acid. The overall difference in selectivity between the extraction techniques also highlights the need for multiple extraction techniques in order to obtain as true a representation of the complete volatile profile as possible. This is a simple fact, but often forgotten in volatile research of dairy and other foods. In relation to the four techniques, DI-HiSorb, HS-HiSorb, TD, and HS-SPME, the impact of GC column polarity was far greater than the impact of salting out under the conditions evaluated. It would appear that, unless specifically required to target a VOC (or specific VOCs) using a polar GC column, significantly more VOC information can be attained than using a non-polar GC column. As stated, the impact of salting out was minimal, but did vary depending upon the extraction technique, GC column polarity, and in relation to individual VOCs. Overall, the greatest number of VOCs were extracted by DI-HiSorb using the non-polar GC column, and slightly more without salting out. However, even though the numbers of VOCs extracted by DI-HiSorb was considerably reduced using the polar GC column, the overall abundance of VOCs was higher than achieved with the non-polar GC column. A key element as to why the overall abundances and numbers of VOCs were generally higher with DI-HiSorb, as opposed to the other techniques, was the ability of DI-HiSorb to extract large quantities and volumes of lactones. Only TD failed to extract any lactones in these WMP samples. As HS-HiSorb has the same sorptive PDMS phase as DI-HiSorb, the

different conditions between DI and HS was a key factor influencing the effectiveness of each of these techniques in extracting lactones and other VOCs. It appears that it was more difficult to extract some VOCs using HS than DI, possibly due to their affinity with sample components adversely impacting their phase transition from a liquid to the gas phase during HS analysis, likely exacerbated by higher molecular weight VOCs. It is possible that the importance of lactones in many dairy products may have been underestimated due to the widespread use of HS-SPME DVB/CAR/PDMS and CAR/PDMS phases, where the CAR component may exclude some higher molecular weight lactones (Liberto et al. 2020). Differences in the apparent capacities of the phases associated with the different techniques did not have as much of an impact on VOC extraction as the difference between DI and HS. Differences between dynamic HS (TD) and static HS (HS-HiSorb and HS-SPME) techniques also did not significantly influence VOC extraction in terms of numbers and abundance. The reproducibility of most of the techniques, as assessed by the average relative percentage deviation, were similar, apart from HS-HiSorb, independent of salting out using the polar GC column, which was much diminished. However, reproducibility was very much VOC-dependent and also influenced by salting out and GC column polarity. Thus, in this study, differences between the techniques were impacted more by the choice of DI or HS, phase composition, and GC column polarity than phase capacity or salting out.

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Chapter 5. The impact of pasture and non-pasture diets on the sensory and volatile properties of whole milk powder

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Abstract

This study evaluated the impact of three distinct diets; perennial ryegrass (GRS), perennial ryegrass/white clover (CLV) and total mixed ration (TMR), on the sensory properties and volatile profile of whole milk powder (WMP). The samples were evaluated using a hedonic sensory acceptance test (n = 99 consumers) and by optimized descriptive profiling (ODP) using trained assessors (n = 33). Volatile profiling was achieved by gas chromatography mass spectrometry using three different extraction techniques; headspace solid phase micro-extraction, thermal desorption and high capacity sorptive extraction. Significant differences were evident in both sensory perception and the volatile profiles of the WMP based on the diet, with WMP from GRS and CLV more similar than WMP from TMR. Consumers scored WMP from CLV diets highest for overall acceptability, flavour and quality, and WMP from TMR diets highest for cooked flavour and aftertaste. ODP analysis found that WMP from TMR diets had greater carmelised flavour, sweet aroma and sweet taste, and that WMP from GRS diets had greater cooked aroma and cooked flavour, with WMP derived from CLV diets having greater scores for liking of colour and creamy aroma. Sixty four VOCs were identified, twenty six were found to vary significantly based on diet and seventeen of these were derived from fatty acids; lactones, alcohols, aldehydes, ketones and esters. The abundance of δ-decalactone and δ-dodecalactone was very high in WMP derived from CLV and GRS diets as was γ-dodecalactone derived from a TMR diet. These lactones appeared to influence sweet, creamy, and carmelised attributes in the resultant WMP samples. The differences in these VOC derived from lipids due to diet are probably further exacerbated by the thermal treatments used in WMP manufacture.

Keywords: Whole milk powder, Sensory, Volatile organic compounds, Feeding system

5.1 Introduction

Whole milk powder (WMP) contains 26 to 40% (w/w) fat, and is generally manufactured from raw milk and processed by pasteurisation, concentration, evaporation and spray-drying. WMP is an important ingredient for a wide range of food products as it can be reconstituted to produce milk drinks, infant milk formula, yoghurts, milk chocolate and ice cream, amongst others (USDEC, 2005).

WMP sensory characteristics can be influenced by animal diet, heat treatment and other processing and storage conditions (water activity, moisture, packaging, light and temperature: Baldwin et al., 1991; Birchal et al., 2005; Faulkner et al., 2018; Clarke et al., 2020a). Pasture-based farming systems are widely practiced in Ireland for the majority of lactation, allowing for the creation of a low-cost, animal welfare friendly, natural feed source to produce high-quality milk products, which are considered more organic and healthier by consumers (Whelan et al., 2017). However, feeding concentrates, such as total mixed ration (TMR) and housing cows indoors is widely implemented in most developed countries mainly for economic reasons (Haskell et al., 2006). Numerous studies have explored the composition of milk from different breeds and feeding systems which have demonstrated that pasture derived milks have higher levels of polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) and lower levels of saturated fatty acids compared with those derived from TMR diets (O'Callaghan et al., 2019; Kalač and Samková, 2010). A diet of fresh pasture significantly increases levels of β-carotene, enhancing a yellow colour, particularly obvious in butter products derived from pasture, but also apparent in milk and milk powders. Some studies have shown that volatiles generated indirectly through rumen metabolism from forage can also have a sensory impact on milk. For example, p-cresol probably contributes to the "barn-yard" aroma/flavour of cow milk

derived from pasture (Kilcawley et al., 2018; Faulkner et al., 2018) and it is likely that some volatiles are directly transferred from diet (Villeneuve et al., 2013).

Thermal processing conditions can also alter the volatile profile of milk products. For example, increases of aldehydes and methyl ketones derived from lipid oxidation (decarboxylation or light induced oxidation) of β-keto acids after heat treatment (Vazquez-landaverde et al., 2006; Hougaard et al., 2011), sulphur compounds formed by oxidation of methanethiol (Contarini et al., 1997), esters from heat-catalysed esterification reactions (Faulkner et al., 2018), increases in γ-lactones derived from hydroxy fatty acids after heat processing (Yoshinaga et al., 2019), increases in ketones formed by β-oxidation of saturated fatty acids (Li et al., 2012), Maillard reaction products such as benzaldehyde, furans, maltol, acetaldehyde, 3-methylbutanal, 2-methylbutanal, 2-methylpropanal and possibly acetophenone (Calvo and de la Hoz, 1992) and the degradation of β-carotene resulting in the formation of toluene and xylenes (Zepka et al., 2014). Several ketones, aldehydes and sulphur compounds have been reported to increase during ultra-high temperature treatment and sterilization of milk (Soukoulis et al., 2007; Al-Attabi et al. 2009; Zabbia et al., 2012). Many of these VOCs can be perceived as off-flavours and can be problematic in products such as WMP, and potentially even carry through into the final product applications resulting in consumer complaints (Hough et al., 2002). Storage time was also shown to have an effect on losses of VOCs in milk powders and this could be due to metabolic and enzymatic reactions post pasteurization, or chemical reactions (Contarini et al., 1997; Clarke et al., 2020b).

Therefore, VOC and sensory profiling of milk and milk products, especially powders such as WMP, is necessary for quality and shelf life purposes. Sensory profiling when undertaken in association with VOC analysis can provide useful

additional information in relation to the association between VOCs and sensory properties. VOC profiling is predominately undertaken using gas chromatography mass spectrometry (GC-MS) and recent studies have shown the importance of extraction method choice in relation to VOC profiling of WMP (Cheng et al., 2021) and the impact of diet on the oxidative and sensory shelf life of WMP (Clarke et al., 2021). Three distinct VOC extraction techniques were utilised in this study in order to obtain a more complete VOC profile; headspace solid phase micro-extraction (HS-SPME), thermal desorption (TD) and a high capacity sorptive extraction (Hi-Sorb) procedure by direct immersion (DI). The aim of this study was to investigate the effect of three distinct diets (GRS, CLV and TMR) on the sensory and VOC profile of WMP.

5.2 Materials and Methods

5.2.1 Whole Milk Powder Manufacture

Raw milk from 54 Friesian cows was split into 3 groups (n=18) at the Teagasc Moorepark dairy farm, Teagasc, Animal & Grassland Research Centre, Fermoy, Co.Cork, Ireland. Each group of 18 cows were given separate diets; outdoor pasture grazing on perennial ryegrass (Lolium perenne) denoted as GRS, outdoor pasture grazing on perennial ryegrass supplemented with white clover (*Trifolium repens*) denoted as CLV, and indoors on TMR consisting of grass silage, maize silage and concentrates, which was a replication of the study outlined in detail by O'Callaghan et al. (2016). Raw whole milk (approximately 1000 kg) was collected from cows on each dietary treatment. The preparation of the WMP was as outlined in Cheng et al. (2021). The milk was not standardised, but the average fat contents of the milk from each diet in triplicate were quite similar (GRS 27.9%, CLV 28.2%, TMR 28.2%). WMP samples were stored at room temperature in sealed 900g aluminium vacuum

cans until analysis (all analysis was undertaken within the designated shelf life of the samples).

5.2.2 Sample Preparation

Prior to sensory and VOC analysis WMP from each dietary treatment (GRS, CLV and TMR) at <2 months was dissolved at 10% solids (w/v) using ultra-pure deionized water and stored at 4°C overnight to ensure solubility without overhead lights to prevent light-induced off-flavour formation as outlined in Cheng et al. (2020) in the preparation of SMP for sensory analysis. VOC analysis and sensory analysis were performed the following day.

To monitor the performance of each extraction procedure, an internal standard (IS) of 2-phenyl-D5-ethanol and 4-methyl-2-pentanol (Merck, Arklow, Wicklow, Ireland) at 20 mg L^{-1} in ultra-pure water, was added (50 µL) to each WMP sample prior to extraction.

5.2.3 Volatile Organic Compound Extraction

The extraction procedures for HS-SPME, TD and DI-HiSorb were identical to those described by Cheng et al. (2021).

5.2.4 GC-MS Analysis

The GC-MS system was an Agilent 7090A GC and Agilent 5977B MSD (Agilent Technologies Ltd., Cork, Ireland) using a non-polar column DB5 (60 m x 0.25 mm x 0.25 µm) (Agilent Technologies Ltd., Ireland). The column oven was held at 40 °C for 5 min, then increased to 230°C at 5 °C min^{-1} and held at 230 °C for 35 min, yielding a total run time of 60 min. The carrier gas was helium held at a constant flow of 1.2 ml min^{-1}. The ion source temperature was 220°C and the interface

temperature was set at 260°C. The mass spectrometer was in electronic ionization (70v) mode with the mass range scanned between 35 and 250 amu. Analysis was undertaken using Mass Hunter Qualitative Analysis Software (Agilent Technologies Ltd) with target and qualifier ions and linear retention indices for each compound compared an in-house library based on mass spectra obtained from NIST 2014 mass spectral library MS searching (v.2.3, Gaithersburg, MD, USA), and an in-house library created using authentic compounds with target and qualifier ions and linear retention indices for each compound using Kovats index. Spectral deconvolution was also performed to confirm identification of compounds using Automated Mass spectral Deconvolution and Identification System (AMDIS). Batch processing of samples was carried out using MetaMS (Wehrens et al., 2014). MetaMS is an open-source pipeline for GC–MS-based untargeted metabolomics. The peak areas of the analytes were normalized to the peak areas of the IS (4-methyl-2-penanol) at first and then expressed as a percentage of the total.

To monitor the performance of the GC-MS operating conditions, an external standard (ES) solution was added at the start and end of each GC-MS sample run, the peak areas were monitored to ensure they were within a specified tolerance (10% coefficient of variation) to ensure that both the SPME extraction and MS detection were performing within specification during the analysis. The ES was comprised of 1-butanol, dimethyl disulfide, butyl acetate, cyclohexanone, and benzaldehyde (Merck, Ireland) at 10 mg L^{-1} with 2-phenyl-D5-ethanol (Merck, Arklow, Co., Wicklow, Ireland) added at 5 mg L^{-1} in ultra-pure water. For the HS-SPME technique, 10 µL of ES was added to the sample in a 20 mL amber HS- SPME vial (Apex Scientific Ltd., Maynooth, Ireland). For TD and DI-HiSorb, the ES (10 µL) was added to the TD tube containing the sample extract for both TD and DI-HiSorb extracts.

5.2.5 Sensory Analyses

All sensory analysis was undertaken at the sensory facility within the School of Food and Nutritional Science, University College Cork, Co. Cork, Ireland, according to International Standards (ISO 11136, 2014).

Milk consumers residing in Cork (Ireland, n=99) (70:30 male/female, age 18-50 years), participated in the consumer test (hedonic attribute testing). The consumers consisted of students and staff from Sensory Group, School of Food and Nutritional Science, University College Cork, Co. Cork, Ireland. Consumers were regular self-reported consumers of milk, had experiences in drinking powdered milk products, and were non-rejecters of milk. Participants used the sensory hedonic descriptors (Table 5.1) provided to them for three different WMP samples (CLV, GRS and TMR) presented in triplicate at 10% solids (w/v). For consumer testing, samples were dispensed into 30 mL inert plastic tumblers provided with three digit codes presented simultaneously but with randomised order to prevent first order and carry-over effects (Macfie et al., 1989). Samples were taken from the refrigerator (4°C) and served after 15 min at 12°C temperature. Participants were first asked to evaluate the overall appearance and colour of the sample. The appearance of each sample was scaled using a 1–9 hedonic scale, where 1 = dislike extremely and 9 = like extremely. They were then asked to taste the sample and evaluate their overall impression using a 9-point hedonic scale. The assessors were asked to assess using a 9-point hedonic scale the liking of flavour, freshness, liking of appearance, liking of aroma, overall acceptability, cooked flavour, thickness, creaminess, aftertaste, intensity of aftertaste and quality.

Optimized descriptive profiling (ODP: da Silva et al., 2012; da Silva et al., 2013) was undertaken with trained panellists at University College Cork, Ireland (n =

33). These assessors were presented with all samples simultaneously, but with randomised order to prevent first order and carry-over effects (Macfie et al., 1989). Assessors used the consensus list of sensory descriptors as described by Cheng et al. (2020) which were measured on a 10 cm line scale with the term “none” used as the anchor point for the 0 cm end of the scale and “extreme” for the 10 cm end of the scale (Table 1). Sensory terms, which were the main sensory dimensions, were pre-selected from the sample set using an expert sensory panel (n = 10). Assessors evaluated the intensity of each attribute for each sample on the scales. Attributes were presented along with the table describing the sensory terms (Table 5.1). All samples were prepared in the same manner as the consumer analysis study and presented in duplicate.

Table 5.1 Sensory terms for the affective (consumer acceptance testing) and optimized descriptive profiling (ODP) of whole milk powder

Descriptor	Explanation	Scale
Consumer Acceptance Testing		
Appearance-Liking	The liking of appearance	0 = extremely dislike 10 = extremely like
Flavour-Liking	The liking of flavour	0 = extremely dislike 10 = extremely like
Aroma-Liking	The liking of aroma	0 = extremely dislike 10 = extremely like
Texture-Liking	The liking of texture	0 = extremely dislike 10 = extremely like
Overall acceptability	The acceptability of the product	0=extremely unacceptable 10 = extremely acceptable
Colour-Liking	The liking of colour	0 = extremely dislike 10 = extremely like
Optimized Descriptive Profiling		
Appearance-colour	Appearance-Ivory to orange colour	0 = Pale, 10 = Yellow
Sweet aroma	The smell associated with dairy sweet milky products	0 = none, 10 = extreme
Creamy aroma	The smell associated with creamy/milky products	0 = none, 10 = extreme
Cooked aroma	The smell associated with cooked milk products	0 = none, 10 = extreme
Oxidised aroma	The smell associated with rancid or oxidised products	0 = none, 10 = extreme
Painty aroma	The smell associated with rancid paint type notes	0 = none, 10 = extreme
Chalky Texture	Chalk like texture in the mouth	0 = none, 10 = extreme
Powdery Texture	Powdery texture in the mouth	0 = none, 10 = extreme
Viscosity	Thick texture in the mouth	0 = none, 10 = extreme
Sweet taste	Fundamental taste sensation of which sucrose is typical	0 = none, 10 = extreme
Cream flavour	The flavour associated with creamy/milky products	0 = none, 10 = extreme
Dairy sweet flavour	The flavours associated with sweetened cultured dairy products such as fruit yoghurt	0 = none, 10 = extreme
Dairy fat flavour	Intensity of fat flavour	0 = none, 10 = extreme
Oxidised flavour	The flavour associated with rancid or oxidised products	0 = none, 10 = extreme
Rancid butter	The flavour associated with rancid or oxidised butter	0 = none, 10 = extreme
Painty Flavour	The flavour associated with rancid paint type notes	0 = none, 10 = extreme
Fruity/Estery flavour	The flavours associated with fatty acid ethyl esters	0 = none, 10 = extreme
Cooked flavour	The flavour associated with cooked milk products	0 = none, 10 = extreme
Off-flavour	Off-flavour (Rancid)	0 = none, 10 = extreme
Astringent after-taste	Fundamental taste sensation of which aluminium sulphate is typical	0 = none, 10 = extreme

5.2.6 Statistical Analysis

Statistical analyses for data relating to volatile analysis were carried out using one way-ANOVA. The level of significance for correlation was set at $P < 0.05$. To classify WMP samples in a supervised multivariate model, partial least squares discriminant analysis (PLS-DA) was performed. Unsupervised hierarchical clustering analysis (HCA) was used to show the patterns in the VOC profile and is presented as a heatmap (Online Supplementary Figure S1). Consumer acceptance data obtained from sensory analysis was evaluated by one-way ANOVA using differences of perception related to diet as the primary factors. Analyses were carried out at only one time point and where normally distributed were analysed using one-way ANOVA with post hoc. The SPSS V23.0 (IBM Statistics Inc., Armonk NY) was used for one-way ANOVA. Principal component analysis (PCA) plots of the volatile and diet data were used to demonstrate correlations between the VOC and the different diet attributes. These were constructed using the "factoextra" and "FactoMinoR" packages in R (v 3.4.1). The Unscrambler X software, version 10.3 (Camo Software, Oslo, Norway) was used for ANOVA-PSLR (APLSR) analysis of WMP data from different diets and variance of OPD sensory data. Analysis of variance (ANOVA) with post hoc Tukey significant test was applied to OPD data, working at an alpha level of 0.05. The correlations between sensory attributes and VOC were also analysed by PLSR. PLSR were performed with VOC data as the X-matrix and sensory attributes as the Y-matrix. Regression coefficients were analysed by Jack-knifing to derive significance indicators for the relationships determined in the quantitative APLSR (data not shown). Metabolic Analyst 4.0 (McGill University, Montreal, QC, Canada) software was used to perform the HCA graph (Chong et al., 2018).

5.3 Results

5.3.1 Volatile Analysis

The volatile compounds identified in the WMP from the three different diets (GRS, CLV and TMR), using three extraction methods are listed in Table 2. Results are expressed as percentage abundance of each VOC per extraction method based on the recovery in relation to the recovery of the IS for that extraction method. A total of 64 VOCs were identified in these WMP derived from all three diets by HS-SPME, TD and DI-HiSorb. Distinct differences in the VOCs profiles were evident between the different extraction techniques, which highlights the effectiveness of each extraction technique for particular chemical classes. The same trends in relation to individual VOCs were evident across the different extraction techniques where a VOC was identified by one or more extraction technique. Eight lactones, 5 aldehydes, 3 ketones, 3 terpenes, 2 alcohols, 2 esters, 1 acid, 1 hydrocarbon and 1 sulphur compound varied significantly ($P< 0.05$ or $P<0.01$) in the WMP based on cow diet (Table 5.2).

Seven VOCs were significantly more abundant in WMP derived from CLV; 1-pentanol ($P< 0.05$) and 3-methyl-butanal, δ-octalactone, α-pinene, 3-carene, acetic acid and ethyl-benene ($P< 0.01$). Four VOCs were significantly more abundant in the WMP derived from GRS; methyl-hexanoate ($P < 0.05$) and butanal, 2,3-octanedione and 2-nonanone ($P< 0.01$). Five VOCs were significantly more abundant in the WMP derived from TMR; γ-dodecalactone GRS ($P < 0.05$) and dimethyl sulphide, D-limonene, heptanal and o-xylene ($P< 0.01$). Another eight VOCs were significantly more abundant in WMP derived from both CLV and GRS in comparison to WMP derived from TMR; δ-decalactone and δ-dodecalactone ($P< 0.05$) and δ-tridecalactone, hexanol, hexanal, δ-hexalactone, δ-nonalactone and δ-undecalactone ($P< 0.01$). Another VOC, pentanal, was significantly ($P<0.01$) more abundant in WMP

derived from GRS in comparison to WMP from TMR, but was not statistically different to WMP derived from CLV (nor was there any statistical difference between WMP from CLV or TMR). Similarly diacetyl was significantly higher ($P<0.01$) in WMP derived from CLV than from WMP derived from TMR, but was not statistically different to WMP from GRS (WMP from GRS and from TMR were also not statistically different). These associations are more clearly represented in the PCA plots (Figure 5.1A and 5.1B) and by the HCA heatmap (online Supplementary Figure S5.1), where it is evident that both the WMP derived from GRS and CLV diets are more comparable, but distinctly separate from WMP derived from TMR diets (Figure 5.1A and 5.1B).

Table 5.2 The volatile compounds in the whole milk powders derived from different diets, grass (GRS), grass/clover (CLV) and total mixed ration (TMR) by GCMS from three volatile extraction procedures. Results are expressed as percentage abundances for each extraction technique based on the recovery of the internal standard (2-phenyl-D5-ethanol).

			DI-HiSorb				**TD**				**HS-SPME**			
Compounds	CAS no.	REF RI	**CLV**	**GRS**	**TMR**	p-value	**CLV**	**GRS**	**TMR**	p-value	**CLV**	**GRS**	**TMR**	p-value
Alcohol														
Ethanol	64-17-5	426	0.34	0.42	0.05	NS	ND	ND	ND		ND	ND	ND	
1-Pentanol	71-41-0	768	0.65^{a}	0.55^{b}	0.44^{c}	***	3.03^{a}	2.03^{b}	1.13^{c}	***	0.68^{a}	0.61^{b}	0.33^{c}	***
1-Hexanol	111-27-3	868	ND	ND	ND		0.86^{a}	0.67^{a}	0.47^{b}	*	0.71^{a}	0.52^{a}	0.11^{b}	*
1-Hexanol,2-ethyl	104-76-7	1030	1.45	1.36	1.17	NS	8.10	6.94	6.50	NS	ND	ND	ND	
α-Terpineol	10482-56-1	1192	ND	ND	ND		0.26	0.25	0.27	NS	ND	ND	ND	
Aldehyde														
Acrolein	107-02-8	470	1.95	1.59	2.10	NS	ND	ND	ND		ND	ND	ND	
Butanal	123-72-8	596	0.76^{b}	1.53^{a}	0.50^{b}	*	ND	ND	ND		ND	ND	ND	
Butanal, 3-methyl-	590-86-3	654	ND	ND	ND		3.65^{a}	2.47^{b}	2.03^{c}	*	7.99^{a}	5.59^{b}	2.46^{c}	*
Pentanal	110-62-3	697	0.38^{ab}	0.43^{a}	0.21^{b}	*	ND	ND	ND		ND	ND	ND	
Hexanal	66-25-1	801	6.45^{a}	6.21^{a}	2.34^{b}	*	13.65^{a}	11.3^{a}	3.13^{b}	*	10.9^{a}	9.40^{a}	2.97^{b}	*
Heptanal	111-71-7	901	2.54^{b}	2.66^{b}	5.03^{a}	*	20.5^{b}	20.20^{b}	24.98^{a}	*	8.53^{b}	8.45^{b}	12.69^{a}	*
Benzaldehyde	100-52-7	960	0.87	1.11	1.40	NS	1.38	1.31	2.74	NS	0.43	0.34	0.45	NS
Octanal	124-13-0	1004	1.29	1.31	2.40	NS	4.16	2.90	4.22	NS	0.80	0.42	0.51	NS
Benzeneacetaldehyde	122-78-1	1048	0.46	0.43	0.33	NS	ND	ND	ND		ND	ND	ND	
2-Octenal,(E)-	2548-87-0	1057	0.61	0.55	0.42	NS	0.29	0.18	0.43	NS	ND	ND	ND	
Nonanal	124-19-6	1106	6.07	6.20	7.32	NS	17.67	21.10	16.01	NS	1.00	1.04	1.08	NS
2-Nonenal,(E)-	18829-56-6	1160	0.38	0.46	0.45	NS	0.23	0.18	0.19	NS	ND	ND	ND	
Decanal	112-31-2	1205	1.76	1.43	1.38	NS	1.64	2.44	1.18	NS	ND	ND	ND	

(continued)

2-Decenal,(E)-	3913-81-3	1266	0.24	0.31	0.35	NS	0.14	0.14	0.35	NS	ND	ND	ND	
Undecanal	112-44-7	1309	0.21	0.28	0.29	NS	0.21	0.25	0.15	NS	ND	ND	ND	
Dodecanal	112-54-9	1401	0.43	0.56	0.67	NS	0.14	0.27	0.47	NS	ND	ND	ND	
Hydrocarbons														
Benzene	71-43-2	669	1.18	1.55	1.18	NS	6.10	11.62	6.40	NS	2.52	2.27	1.14	NS
Toluene	108-88-3	763	0.35	0.61	0.42	NS	3.97	4.21	2.74	NS	0.40	0.86	0.24	NS
p-Xylene	106-42-3	867	ND	ND	ND		2.52	2.99	3.90	NS	ND	ND	ND	
o-Xylene	95-47-6	900	ND	ND	ND		1.01^{b}	1.09^{b}	1.58^{a}	*	ND	ND	ND	
Phenol	108-95-2	995	0.70	0.67	0.63	NS	0.65	0.70	0.64	NS	ND	ND	ND	
Benzyl alcohol	100-51-6	1037	ND	ND	ND		0.26	0.17	0.25	NS	ND	ND	ND	
Ketone														
Diacetyl	431-03-8	596	ND	ND	ND		0.36^{a}	0.33^{ab}	0.24^{b}	*	ND	ND	ND	
Hydroxyacetone	116-09-6	657	1.36	0.64	1.09	NS	ND	ND	ND		ND	ND	ND	
2-Pentanone	107-87-9	687	1.53	1.41	1.19	NS	6.94	7.70	8.47	NS	5.25	6.29	5.02	NS
2-Butanone	108-10-1	740	ND	ND	ND		7.30	7.10	7.49	NS	1.79	1.35	1.11	NS
Methyl Isobutyl Ketone	108-10-1	740	0.53	0.49	0.43	NS	1.07	0.87	0.84	NS	0.15	0.33	0.23	NS
2-Heptanone	110-43-0	891	6.26	6.28	6.06	NS	7.29	8.92	9.69	NS	17.00	19.1	12.38	NS
2,3-Octanedione	585-25-1	967	0.17^{b}	0.19^{a}	0.11^{c}	*	ND	ND	ND		ND	ND	ND	
2-Octanone	111-13-7	992	0.38	0.38	0.37	NS	0.60	0.36	0.68	NS	ND	ND	ND	
3,5-Octadien-2-one	38284-27-4	1072	ND	ND	ND		0.50	0.60	0.90	NS	0.21	0.10	0.13	NS
2-Nonanone	821-55-6	1094	2.76^{b}	2.83^{a}	2.69^{b}	*	1.31^{b}	1.79^{a}	1.63^{b}	*	2.32^{b}	2.54^{a}	2.13^{b}	*
2-Undecanone	112-12-9	1294	0.47	0.40	0.40	NS	0.16	0.14	0.14	NS	ND	ND	ND	

(continued)

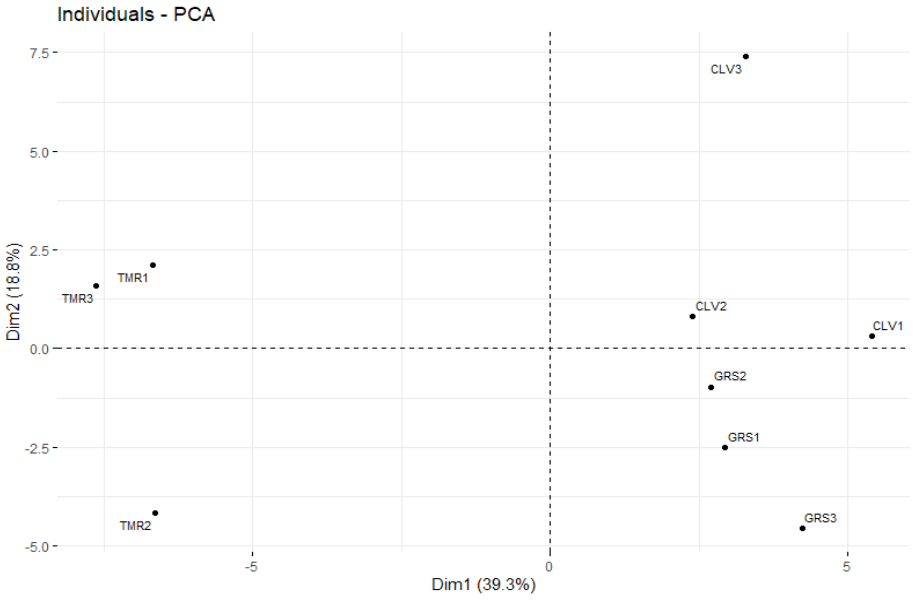
2-Tridecanone	593-08-8	1480	0.34	0.31	0.15	NS	ND	ND	ND		ND	ND	ND	
2-Pentadecanone	2345-28-0	1689	0.55	0.47	0.18	NS	ND	ND	ND		ND	ND	ND	
2-Heptadecanone	2922-51-2	1878	0.56	0.61	0.46	NS	ND	ND	ND		ND	ND	ND	
Lactone														
γ-Crotonolactone	497-23-4	916	0.12	0.27	0.17	NS	ND	ND	ND		ND	ND	ND	
δ-Hexalactone	823-22-3	1084	0.11^{a}	0.12^{a}	0.06^{b}	*	ND	ND	ND		ND	ND	ND	
δ-Octalactone	698-76-0	1288	0.92^{a}	0.75^{b}	0.23^{c}	*	ND	ND	ND		ND	ND	ND	
δ-Nonalactone	3301-94-8	1404	0.15^{a}	0.16^{a}	0.09^{b}	*	ND	ND	ND		ND	ND	ND	
δ-Decalactone	705-86-2	1506	31.00^{a}	32.10^{a}	21.90^{b}	***	ND	ND	ND		0.43^{a}	0.38^{a}	0.28^{b}	***
δ-Undecalactone	710-04-3	1627	0.21^{a}	0.20^{a}	0.08^{b}	*	ND	ND	ND		ND	ND	ND	
γ-Dodecalactone	2305-(05)-7	1674	4.20^{b}	2.80^{c}	34.64^{a}	***	ND	ND	ND		ND	ND	ND	
δ-Dodecalactone	713-95-1	1719	44.50^{a}	45.99^{a}	29.64^{b}	***	ND	ND	ND		ND	ND	ND	
δ-Tridecalactone	7370-92-5	1778	1.45^{a}	1.46^{a}	0.72^{b}	*	ND	ND	ND		ND	ND	ND	
Sulphur compounds														
Dimethyl sulphide	75-18-3	510	ND	ND	ND		ND	ND	ND		1.00^{b}	1.61^{b}	2.42^{a}	*
Dimethyl disulphide	624-92-0	739	0.55	0.51	0.50	NS	5.16	4.21	4.48	NS	0.95	0.41	0.29	NS
Terpenes														
α-Pinene	80-56-8	930	ND	ND	ND		ND	ND	ND		3.28^{a}	1.65^{b}	0.86^{c}	*
3-Carene	13466-78-9	1009	ND	ND	ND		ND	ND	ND		2.92^{a}	1.74^{b}	0.89^{c}	*
D-Limonene	5989-27-5	1022	0.25^{b}	0.25^{b}	0.54^{a}	*	0.53^{b}	0.67^{b}	2.34^{a}	*	0.14^{b}	0.08^{b}	0.37^{a}	*

(continued)

Longifolene	475-20-7	1432	ND	ND	ND		0.23	0.13	0.13	NS	ND	ND	ND	
Acids														
Acetic acid	64-19-7	629	0.37[a]	0.17[b]	0.15[b]	*	ND	ND	ND		ND	ND	ND	
Esters														
Methyl butanote	623-42-7	724	ND	ND	ND		2.02	2.03	1.93	NS	1.81	2.30	3.19	NS
Methyl pyruvate	108-10-1	740	0.12	0.20	0.14	NS	ND	ND	ND		ND	ND	ND	
Ethylbenzene	100-41-4	851	ND	ND	ND		1.15[a]	0.42[b]	0.47[b]	*	ND	ND	ND	
Methy hexanoate	106-70-7	922	ND	ND	ND		ND	ND	ND		1.49[c]	5.45[a]	3.12[b]	***
Furans														
Furan,2-methyl-	534-22-5	604	0.97	0.89	0.90	NS	ND	ND	ND		8.82	7.59	6.35	NS
2-Furanmethanol	98-0-0	850	1.04	0.36	0.38	NS	ND	ND	ND		ND	ND	ND	
Furfural	98-01-1	852	0.56	0.29	0.23	NS	ND	ND	ND		ND	ND	ND	

RI: Retention index. REF RI: Reference retention index. CAS no: Chemical Abstracts Service Number. One-way ANOVA statistical analysis :* and *** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively .Values in the same row not sharing the same superscript (a,b,c) specify significant difference in peak area % value carried out by Tukey post hoc test.

A



B

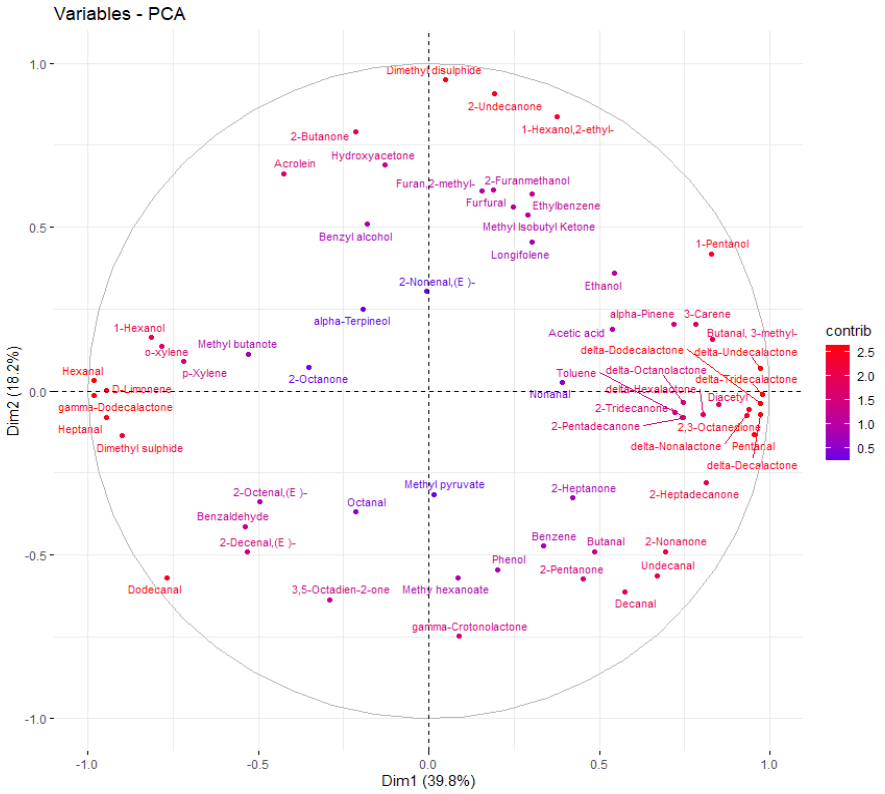
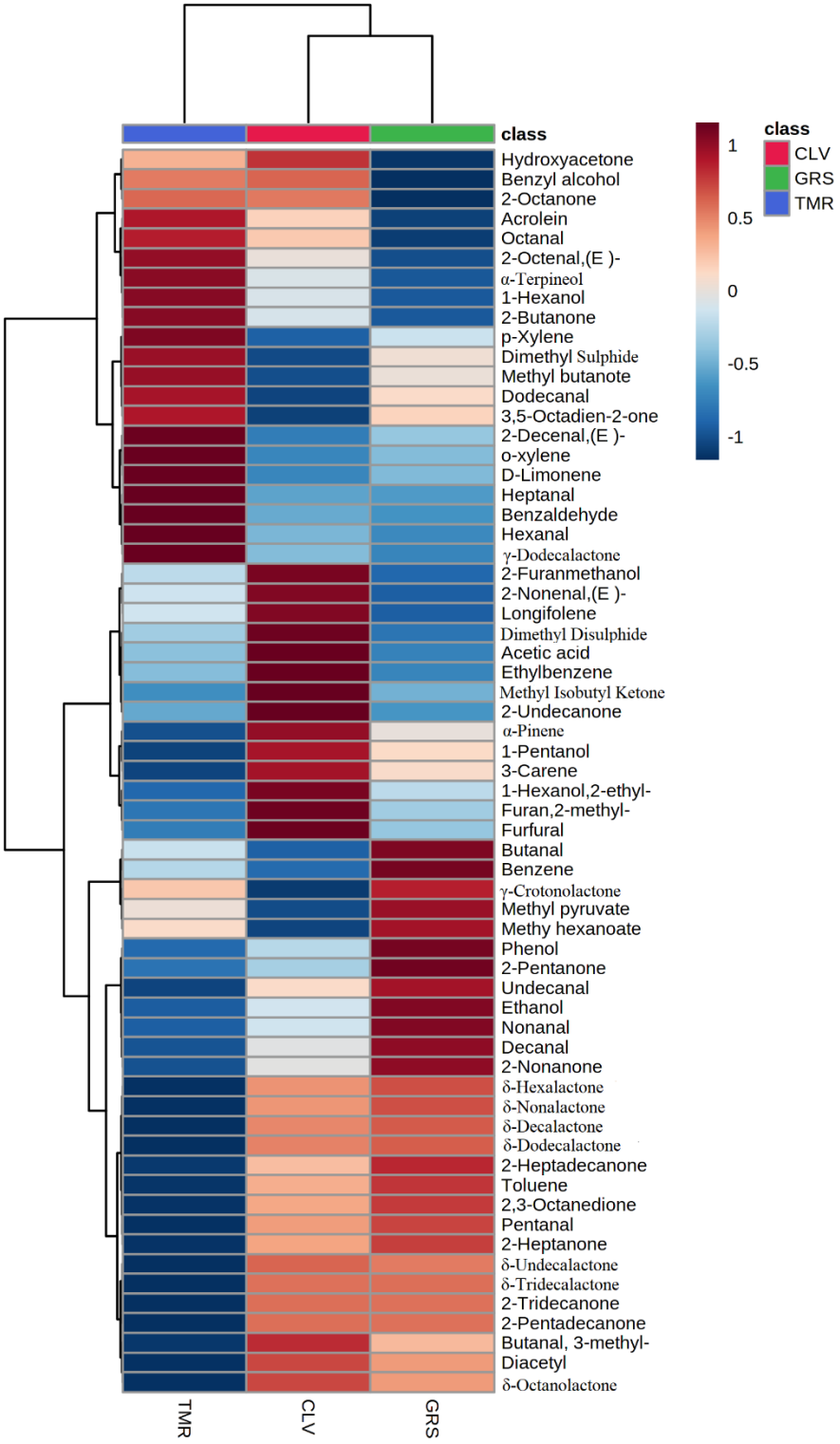


Figure 5.1: Principal component analysis: (a) Three kinds of feeding system of grass (GRS), grass/clover (CLV) and total mixed rations (TMR) .Scores and loadings are based on the average of three repetitions for each feeding diets. (b) Variables: the relative percent amount of 64 volatile compounds. Colour gradient: low = white, mid = blue and high = red, midpoint set 1.0.



Supplementary Figure S5.1 Hierarchical clustering analysis (heatmap) of volatile organic compounds derived from different diets grass (GRS), grass/clover (CLV), and total mixed rations (TMR). The degree of positive and negative correlation is indicated by + 1 (red) to − 1 (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5.3.2 Sensory Evaluation

The sensory attributes identified in the WMP derived from CLV, GRS and TMR diets by consumer acceptance are shown in Figure 5.2. Six significant sensory differences ($P< 0.05$) were observed between the WMP derived from these diets; creaminess, aftertaste, cooked-flavour, quality, overall acceptability and liking of flavour. The WMP derived from CLV diets scored statistically ($P< 0.05$) highest for overall acceptability, liking of flavour, creaminess and quality. The WMP derived from TMR diets scored significantly highest ($P< 0.05$) for cooked-flavour and aftertaste. The WMP derived from GRS diets did not score significantly different for any of the sensory attributes in comparison to WMP samples derived from either CLV or TMR diets.

The ODP evaluation of WMP from different diets is shown in the PCA plot (online Supplementary Figure S5.2). The significance (p-value) of regression coefficients and average results (ANOVA) for the ODP attributes for WMP from different diets (CLV, GRS and TMR) are illustrated in Table 5.3. The assessors rated WMP derived from CLV diets as significantly greater for liking of colour ($P< 0.05$) and creamy aroma ($P< 0.05$) in comparison to the WMP derived TMR diets. The WMP derived from GRS diets scored statistically ($P< 0.01$) higher for cooked aroma and cooked flavour and WMP derived from TMR diets highest for carmelised flavour ($P< 0.05$), sweet aroma and sweet taste ($P< 0.01$).

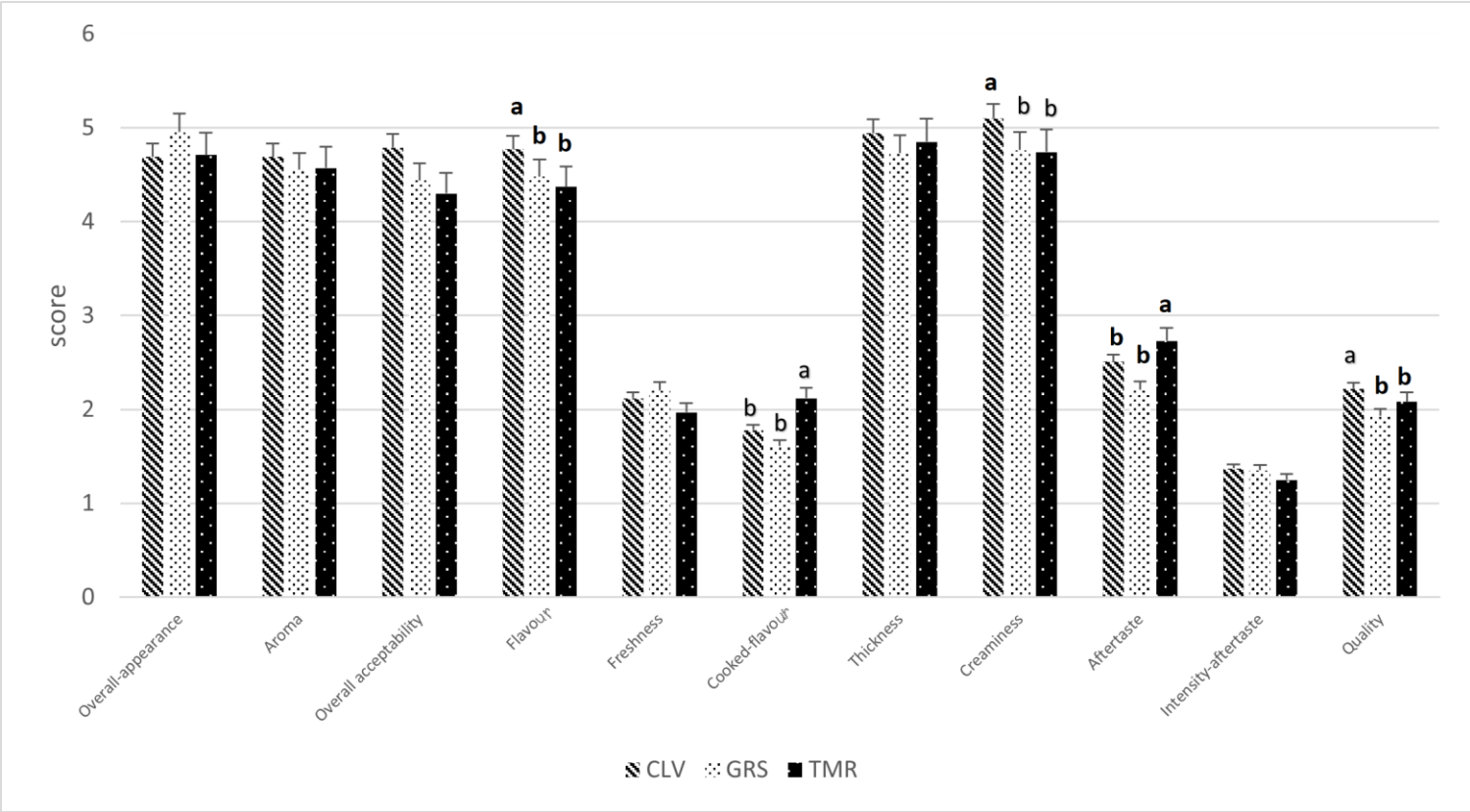
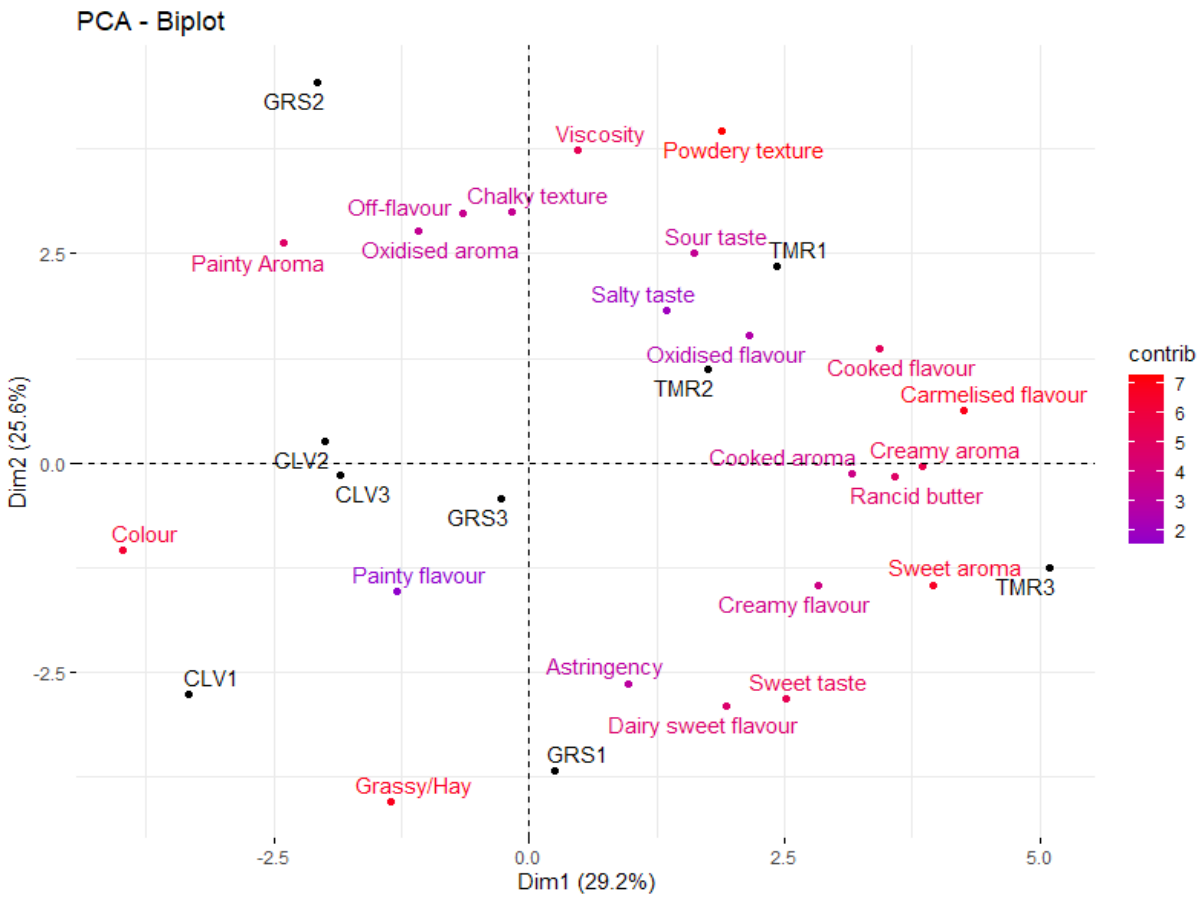


Figure 5.2 Hedonic sensory analysis of whole milk powder drink derived from different feeding systems of grass (GRS), grass/clover (CLV) and total mixed rations (TMR). The whole milk powder samples were assessed by consumers (n = 99) familiar with milk using blind replicates in a full balanced block design, where consumers evaluated all samples in duplicate. The error bars represent standard mean error within replicates. Columns with different letters (a–c) for each attribute are statistically different ($P < 0.05$) carried out by ANOVA and Tukey post hoc test.



Supplementary Figure S 5.2: Optimized Descriptive Profiling. PCA plot for Whole milk powder produced outside on grass (GRS), grass/clover (CLV) or indoors on total mixed rations (TMR) by as evaluated Irish assessors (n = 33).

Table 5.3 ODP (Optimized Descriptive Profiling) evaluation by assessors of whole milk powder produced by different diets, grass (GRS), grass/clover (CLV) and total mixed rations (TMR). Correlation relationships and ANOVA analysis with sensory attributes observed in whole milk powder. Positive and negative values indicate positive and negative correlations between the diets and sensory attributes, respectively.

	Beta Coefficients			**ANOVA**			
	CLV	**GRS**	**TMR**	**CLV**	**GRS**	**TMR**	**P-value**
Colour	0.174***	0.116*	-0.290	5.47[a]	5.78[a]	3.18[b]	***
Sweet aroma	-0.037	-0.025	0.062	4.07[c]	4.57[b]	4.85[a]	*
Creamy aroma	0.038	0.025	-0.064	4.41[a]	4.03[b]	3.72[b]	*
Cooked aroma	0.036	0.024	-0.061	3.74[b]	4.32[a]	3.54[b]	*
Oxidised aroma	0.017	0.011	-0.029	1.86	2.10	1.73	NS
Painty Aroma	0.002	0.001	-0.003	1.38	1.39	1.47	NS
Chalky texture	-0.010	-0.007	0.017	3.06	3.10	3.23	NS
Powdery texture	-0.040	-0.026	0.066	2.99	3.47	3.80	NS
Viscosity	-0.013	-0.009	0.022	4.23	4.19	4.39	NS
Sweet taste	-0.032	-0.021	0.054	5.00[b]	4.97[b]	5.53[a]	*
Sour taste	-0.016	-0.010	0.026	1.94	2.05	2.20	NS
Salty taste	-0.009	-0.006	0.015	1.62	1.48	1.67	NS
Creamy flavour	-0.034	-0.023	0.057	5.12	5.08	5.59	NS
Dairy sweet flavour	-0.015	-0.010	0.024	5.02	4.84	5.18	NS
Carmelised flavour	-0.061*	-0.040 *	0.101	2.95[b]	3.09[b]	4.03[a]	***
Oxidised flavour	-0.012	-0.008	0.020	2.26	2.50	2.53	NS
Rancid butter	-0.021	-0.014	0.036	1.95	2.01	2.27	NS
Painty flavour	0.009	0.006	-0.016	1.64	1.61	1.51	NS
Grassy/Hay	0.014	0.009	-0.023	2.46	2.47	2.27	NS
Cooked flavour	0.005	0.003	-0.008	5.84[ab]	7.67[a]	4.81[b]	*
Off flavour	-0.001	-0.001	0.001	2.08	2.08	2.10	NS
Astringency	-0.009	-0.006	0.014	1.80	1.56	1.80	NS

ANOVA values are the average results. One-way ANOVA statistical analysis :* and *** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively .Values in the same row not sharing the same superscript (a, b,c) specify significant difference in sensory attribute value carried out by Tukey post hoc test.

5.3.3 OPD Sensory and Volatile Data Correlations

APLSR was conducted to study the relationships of the individual VOCs with the sensory descriptors as used in the ODP study. The X-matrix was composed of 64 VOCs, whereas the Y-matrix consisted of the sensory attributes from OPD (Figure 5.3). When the two PLSs were taken into account, 63% of the VOCs explained 36% of the variation among the sensory data and diets. All variances were placed between the inner (r^2 = 0.5) and outer ellipses (r^2 =1.0), thereby indicating that the APLSR model sufficiently described the associations between descriptors and VOCs. The centre ellipsoid in Figure 3 indicates 50% of the explained variation. Many VOC were located inside the ellipsoid, which means they did not greatly contribute to the model. Figure 5.3 demonstrates that the WMP samples are separated along PLS1, with the WMP derived from TMR on the right side and the WMP derived from GRS and CLV on the left side. This highlights again that WMP derived from TMR diets is more distinct than the WMP from both the CLV and GRS diets.

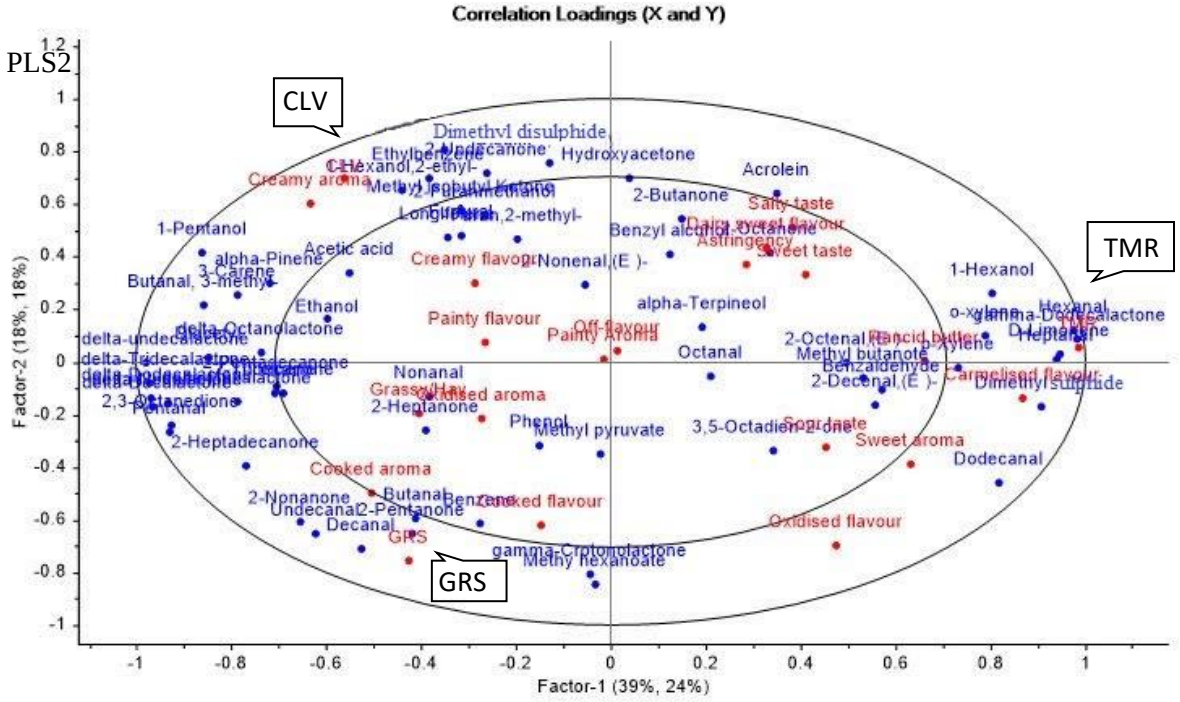


Figure 5.3: ANOVA-PLSR correlation loadings plot of sensory attributes (aroma and flavour) by OPD analysis and volatile compounds (X-matrix) in the WMP from the three distinct diets, grass (GRS), grass/clover (CLV) and total mixed rations (TMR). Ellipses represent r^2 =0.5 and 1.0, respectively.

5.4 Discussion and Conclusion

The source of many of these VOCs in WMP is varied and probably a combination of some or all of the following; direct transfer from the diet, rumen metabolism, metabolism in the raw milk, created during heat processing or by auto chemical reactions. However, some trends were evident. Seventeen of the twenty six VOCs that were significantly different based on diet in these WMP samples are derived from fatty acids either by lipid oxidation, thermal degradation or β-oxidation and lactonization (butanal, pentanal, hexanal, hexanal, 1-pentanol, 1-hexanol, 2-nonanone, 2,3-octanedione, δ-hexalactone, δ-octanolactone, δ-nonalactone, δ-decalactone, δ-undecalactone, δ-dodecalactone, δ-tridecalactone, γ-dodecalactone). It is well established that different diets have a significant impact on the fatty acid profile of cows milk (Kalač and Samková, 2010; O'Callaghan et al., 2016), hence it is logical that VOCs derived from fatty acids are also likely to be significantly impacted. Such differences may be further exacerbated by subsequent thermal treatment during processing to WMP enhancing lipid oxidation and Maillard and lactonization reactions (Calvo and de la Hoz, 1992; Havemose et al., 2006; Kilcawley et al., 2018; Clarke et al., 2021).

Straight-chain aldehydes are major contributors to off-flavours in dairy products (Barrefors et al., 1995). Previous studies have also found that the abundance of methyl ketones also from lipid oxidation were correlated to the severity of heat treatment in milk and associated with off-flavour development that can be carried over to final product applications (Nursten, 1997). Only one methyl ketone, 2-nonanone was significantly different based on diet, and most abundant in WMP derived from GRS. The ketone 2,3-octanedione, which is also a product of lipid oxidation, has

previously been shown to be higher in milk derived from pasture (Coppa et al., 2011) as found in this study. Two primary alcohols, 1-pentanol and 1-hexanol are also products of lipid oxidation. 1-Pentanol is derived from the primary aldehyde pentanal and was significantly correlated with the WMP from CLV diets, in agreement with previous studies on milk (Faulkner et al., 2018; Clarke et al., 2020a). Villeneuve et al. (2013) found levels of 1-pentanol were significantly higher in milk derived from pasture and silage than from hay, however, other studies have not found an impact of diet on levels of 1-pentanol (Coppa et al., 2011). 1-Hexanol, derived from hexanal (Zhang et al., 2016) was significantly higher in the WMP derived from TMR diets, in agreement with that found by Faulkner et al. (2018) and Clarke et al. (2020b) in milk, but other studies did not find an impact of diet on levels of 1-hexanol in milk (Villeneuve et al., 2013). Esters are probably present as a result of microbial activity in the rumen, post-pasteurisation microbial contamination or heat-catalysed esterification during thermal-treatment (Whitfield et al., 2000; Kilcawley et al., 2017). Only two fatty acid esters were found in these WMP samples and only methyl hexanoate varied with diet. Methyl hexanoate was found to be significantly more abundant in WMP derived from GRS diets.

The significant difference in the abundance and presence of lactones in the WMP derived from these diets is especially interesting due to their potential positive sensory impact (the abundance of 8 of the 9 lactones identified varied with diet). Lactones are cyclic compounds, formed through metabolism and/ or thermal degradation of δ- and γ-hydroxyacids and are known to contribute to sweet, creamy and fatty flavours in milk powders (Yoshinaga et al., 2019; Ianni et al., 2020). The recovery of lactones was mostly achieved using the DI-HiSorb extraction method, which is a sorptive extraction technique particularly useful for extracting lactones in

contrast to other extraction methods (High et al., 2019; Cheng et al., 2021). Some studies have stated that milk from cows fed hay (timothy) was characterized by higher contents of γ-lactones than silage-fed and pasture-fed derived milk (Villeneuve et al., 2013; Bovolenta et al., 2014). This may be why γ-dodecalactone is significantly more abundant in WMP from TMR in this study. The proliferation of both δ-decalactone and δ-dodecalactone in these samples, but especially in WMP derived from CLV and GRS diets, might also relate to changes in the fatty acid profile and hydroxy acid content due to the different diets (Villeneuve et al. 2013; Ianni et al., 2020). As thermal treatments increase the levels of free hydroxy acids it may be assumed that dietary differences in lactone contents are exacerbated during the spray drying process for the preparation of the WMP (Calvo and de la Hoz, 1992).

The remaining VOC that were significantly different based on diet and not derived from lipids were 3-methyl-butanal, dimethyl sulphide, α-pinene, 3-carene, D-limonene, ethylbenzene, o-xylene, acetic acid, and diacetyl. The only Strecker degradation product was 3-methyl-butanal which was significantly more abundant in WMP derived from CLV diets and is a product of leucine metabolism, or also possibly directly transferred from the diet (Faulkner et al., 2018). Strecker derived VOCs can also be involved in the Maillard reaction, and some have previously been shown to be indicators of severely heat-treated milk and UHT milk (Calvo and de la Hoz, 1992; Belitz et al., 2004). Previous studies have identified 3-methyl-butanal in milk, but it was not impacted by diet (Bendall, 2001; Faulkner et al., 2018). Dimethyl sulphide was significantly higher in WMP derived from TMR than from GRS or CLV diets. Dimethyl sulphide is mainly derived from methionine, but the impact of diet is unclear (Clarke et al., 2020b), possibly because many sulphur compounds are so reactive and thermally labile (Vazquez-Landaverde et al., 2006; Hougaard et al., 2011). Zabbie et

al. (2012) also highlighted that Maillard reactions during heat treatment of milk also generate sulphur- and nitrogen-containing compounds. It is likely that any potential differences due to diet may also be exacerbated by thermal-treatments during the production of the WMP.

Only a minority of terpenes or VOC derived from carotenoids were present in these WMP samples. Terpenes, are directly transferred from feed (Kalač, 2011), but also generated through metabolism of sesquiterpenes in rumen or in milk (Fedele et al., 2004). The monoterpenes, α-pinene and 3-carene were significantly more abundant in WMP derived from CLV diets, and D-limonene was significantly more abundant in WMP derived from TMR. Previous studies have highlighted changes in terpene content due to diet and season (Prache et al., 2005; Engel et al., 2007). o-Xylene is also potentially derived from β-carotene degradation or possibly from direct transfer from diet (Kilcawley et al., 2018). In this study, o-xylene was significantly higher in WMP derived from TMR diets. Ethylbenzene, another product of carotenoid degradation (Zhang et al., 2016) was significant higher ($P< 0.01$) in WMP derived from a CLV diet, but a previous study did not show any impact of diet on ethylbenzene levels in milk (Coppa et al., 2011).

WMP derived from CLV had significantly more acetic acid compared to WMP from either GRS or TMR diets. Acetic acid is primarily a product of carbohydrate metabolism or can be produced from the metabolism of amino acids, but is also known to be directly transferred from feed (Kilcawley, 2017; Clarke et al., 2020b). Most studies have found acetic acid in milk, but the dietary impact varies (Croissant et al., 2007; Villeneuve et al., 2013). Diacetyl was significantly more abundant in WMP derived from GRS diets. Diacetyl is widely found in milk and is a result of pyruvate metabolism or direct transfer (Moio et al., 1996).

In terms of consumer acceptance testing, creaminess, aftertaste, cooked-flavour, quality and flavour were significantly different in these WMP based on diet. WMP from CLV was significantly higher for quality, which is probably related to the fact that it was also highest for creaminess and flavour, but significantly lowest for cooked flavour, and aftertaste. Diet is known to influence the composition and distribution of fat and protein in milk, which can impact creamy and smooth perception (Frøst and Janhøj, 2007). O'Callaghan et al. (2016) found that milk derived from a CLV diet had a higher level of fat and protein than milk derived from a TMR diet. In this study, WMP derived from CLV diets scored significantly higher for creaminess, which is probably related to the impact of diet on fat content and distribution. Clarke et al. (2020b) also found similar results where a trained descriptive panel scored creaminess significantly higher in pasteurised milk derived from CLV diets in comparison to milk derived from GRS and TMR diets. It is also plausible that the very abundant lactones (δ-decalactone, δ-dodecalactone and γ-dodecalactone) that differed due to diet also impacted creamy perception (Karagül-Yüceer et al., 2001; Villeneuve et al., 2013). It is difficult to explain a relationship between aftertaste in WMP and the impact of diet, although it may be associated with cooked flavour as both were significantly higher ($P<0.05$) in WMP derived from TMR diets. The development of cooked/milky flavour in UHT milk has been suggested to be derived from milk protein denaturation, particularly serum protein, and possibly also linked to volatile sulphur compounds (Deeth, 2010; Zabbia et al., 2012). However, as mentioned, only dimethyl sulphide was found to be significantly different ($P<0.01$) in this study based on diet, but at greatest abundance in WMP derived from TMR.

The ODP assessors found a significant impact of cow diet (CLV, GRS and TMR) on WMP for colour, carmelised flavour, sweet aroma, sweet taste, creamy

aroma, cooked aroma and cooked flavour. WMP derived from CLV and GRS diets was significantly higher for liking of colour compared to WMP derived from TMR diets (Table 3). This result is similar to that reported by Faulkner et al. (2018) for pasteurised milk, and due to higher β-carotene contents. The correlation of the colour of dairy products and β-carotene content has been extensively highlighted and reviewed by Martin et al. (2005). ODP data also clearly identified that both sweet aroma, and sweet taste scored significantly higher for WMP derived from TMR diets. This result is similar to that obtained by Villeneuve et al. (2013) who found sweet flavour higher in milk from cows fed hay as opposed to pasture and silage, as did Croissant et al. (2007), when comparing milk from Jersey and Holstein cows fed TMR versus pasture. Villeneuve et al. (2013) speculated that this may be due to the abundance of γ-lactones, which corresponds well with this study where γ-dodecalactone was present at significantly higher abundances in WMP from TMR and is characterised with a sweet, green odour (Karagül-Yüceer et al., 2001). Carmelised flavour was also significantly higher in WMP derived from TMR diets and may also be linked to Maillard reactions (Kilcawley et al., 2018), and/ or to the greater abundance of γ-dodecalactone. Both cooked aroma and cooked flavour were significantly higher in WMP derived from a GRS diet compared to WMP from TMR and CLV diets. This is different to that found in the consumer acceptance part of this study, and in previous studies (Clarke et al., 2020b; Manzocchi et al., 2021). The only VOC significantly higher in just GRS WMP were butanal and methyl hexanoate neither of which are associated with cooked flavour. The significant difference in creamy aroma which was higher in WMP derived from CLV than from TMR and GRS diets, similar to that found in the consumer part of this study. As mentioned, this may relate to differences in fat particle size distribution, fat globule flocculation or fat

coalescence due to the presence of lower-melting-point fatty acids and lactones due to diet (Richardson et al., 1993; Villeneuve et al., 2013; O'Callaghan et al., 2016; Faulkner et al., 2018). However, previous studies have not found that diet impacted creamy perception in milk (Monzocchi et al., 2021).

The WMP derived from GRS diets was positively correlated to cooked aroma and with butanal, 2-pentanone, decanal, undecanal, 2-nonanone, γ-crotonlactone, methyl hexanoate and 2-heptadecanone. It is difficult to associate these individual VOCs with cooked aroma as none have previously been directly associated with this attribute. The WMP derived from CLV diets was positively correlated with creamy aroma, dimethyl disulphide, 2-undecanone, ethyl benzene, 2-ethyl-1-hexanol, 1-pentanol, α-pinene, 3-carene and 3-methyl-butanal. None of these VOCs would typically be associated with cream attributes. However, WMP derived from both GRS and CLV were also associated with many δ lactones that are probably influencing the creamy aroma, especially as some were very abundant. The WMP derived from TMR diets was positively associated with carmelised flavour, sweet aroma and oxidised flavour, but also with dimethyl sulphide, o-xylene, p-xylene, hexanal, 1-hexanol, γ-dodecalactone, D-limonene, heptanal and dodecanal (obtained from the jack-knife uncertainty test, Fig 3). Sweet taste has previously been found to be a dominant sensory descriptor for WMP derived from TMR diets (Clarke et al., 2021) and sweet has also been associated with concentrate diets, like TMR and γ-lactones (Villeneuve et al., 2013). Chen et al. (2017) also found that greater abundances of γ-lactones corresponded with higher scores for the sweet, vanilla and caramel descriptors.

In conclusion, significant differences were evident in the sensory perception and volatile profile of the WMP based on the diet. Consumers rated the overall acceptability, flavour, creaminess and quality of the WMP derived from CLV higher than WMP derived from GRS or TMR diets. Consumers also found that WMP derived from TMR diets scored higher for cooked flavour and aftertaste. The familiarity of the consumers in this study with pasture derived dairy products maybe a factor influencing these results. ODP analysis with trained assessors found that colour was one of the most discriminatory sensory attributes for these WMP based on diet and was significantly positively correlated to WMP derived from CLV diets. Overall ODP analysis found that WMP from all three diets differed, but that WMP from both pasture diets (CLV and GRS) were more closely related, which was confirmed by VOC analysis. The different VOC extraction techniques used provided a more comprehensive volatile profile of these WMP samples, with sixty four individual VOCs identified. Similar trends were evident for those VOCs which were extracted by each technique in relation to the impact of diet. However, DI-HiSorb extracted significantly more VOCs than TD or HS-SPME, but was especially beneficial for extracting lactones. This proved to be very useful as the abundance of some δ-lactones (δ-decalactone and δ-dodecalactone) and γ-dodecalactone were very high. Both δ-decalactone and δ-dodecalactone were significantly more abundant in WMP derived from CLV and GRS diets, with the abundance of γ-dodecalactone significantly higher in WMP from TMR diets. γ-Dodecalactone was probably influencing the sweet aroma and flavour and possibly carmelised flavour associated with WMP from TMR, with δ-decalactone and δ-dodecalactone probably influencing the positive association of creamy aroma with the WMP from the CLV diet. ODP analysis did not find any negative sensory attributes associated with lipid oxidation in these samples, and this

is probably due to the fact that they were analysed not long after manufacture, therefore, potential differences did not have sufficient time to develop. It was also apparent that by far most of the significant differences in relation to VOCs in these samples in relation to diet were those primarily derived from fatty acids; lactones, primary and secondary aldehydes, alcohols, ketones and an ester. In hindsight this is not surprising as diet has such a significant impact on the fatty acid profile of milk and is, therefore, likely to impact on subsequent VOCs from this source. This study has also highlighted that differences in VOC due to diet are probably exacerbated by thermal treatment and thus another important contributory factor in WMP manufacture.

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Chapter 6. Optimization of a direct immersion high capacity sorptive extraction gas chromatography mass spectrometry method to determine the impact of cow feeding systems on the volatile and aromatic properties of salted butter

Abstract

The impact of bovine diet on the volatile and aromatic properties of salted butter is of interest for authentication, sensory and marketing purposes. In order to gain this information a suitable volatile extraction method is required to capture the wide range of volatile organic compounds (VOC). Thus in this study a novel volatile extraction technique direct immersion high capacity sorptive (DI-HiSorb) extraction was optimized in tandem with gas chromatography mass spectrometry and olfactometry analysis. Salted butter was prepared from 54 cows split into three groups of 18 cows on three distinct diets; outdoors on perennial rye grass (GRS), outdoors on perennial rye grass and white clover (CLV), and indoors on a total mixed ration (TMR). This study has demonstrated that the optimized and validated DI-HiSorb GC–MS method was very suitable for the detection of VOC in salted butter and for olfactory analysis. In total 66 VOC were identified and the abundance of 40 were significantly ($p < 0.05$) impacted by diet. Three of the most discriminating VOC in these salted butter samples based on diet were lactones (γ-octalactone, γ-6-(Z)-dodecenolactone and γ-dodecalaceone), which are derived from lipid hydroxy acids. Thirty eight distinct odors were perceived, with 24 positively identified. Most odors were perceived in all three salted butter samples from all diets, although the perception of 7 were impacted by diet. This study has highlighted the benefit of DI-HiSorb for volatile analysis of salted butter and as a suitable extraction technique for olfactory anlaysis of salted butter.

Keywords: Butter, Diet, Olfactometry, Aroma

6.1 Introduction

Salted butter is widely consumed all over the world, the sensory properties of which are impacted by cow diet, season of production, manufacturing process and storage conditions (Shooter et al. 1999; Peterson and Reineccius, 2003; Couvreur et al. 2006; Panseri et al. 2011). Pasture-based farming systems are widely practiced in Ireland for the majority of lactation, allowing for the creation of a low-cost, animal welfare friendly, natural feed source to produce high-quality milk products, often considered more organic and healthier by consumers (Whelan et al. 2017). The impact of pasture-based feeding systems vs. feeding concentrates is known to significantly impact the fatty acid profile of milk (O'Callaghan et al. 2016), volatile and sensory properties of subsequent dairy products (O'Callaghan et al. 2016; Garvey et al. 2020; Cheng et al. 2020).

Previous studies have identified a range of volatile organic compounds (VOC) that impact on the sensory properties of butter; acids, aldehydes, ketones, lactones, alcohols, esters, thiazoles and sulphur compounds (Widder et al. 1991; Guth and Grosch, 1992; Peterson and Reineccius, 2003; Mailla et al. 2008a; Mailla et al. 2009; Li et al. 2020) by gas chromatography mass spectrometry (GC-MS). Literature suggests that aldehydes, lactones, short chain free fatty acids and ketones are the most abundant VOC in salted butter. This is not surprising as salted butter contains ~80% milk fat and most of these VOC are derived from fatty acids. A recent study of salted butter produced from cows on pasture diets indicated that some aldehydes, ketones and acids are possibly influencing grassy, milky and sour flavours, and buttery aroma as perceived by consumers and assessors in Germany, USA and Ireland (Garvey et al. 2020). Another notable factor is that virtually all of these fat derived chemical classes, except acids are also compounded by increases in temperature, thus indicating the

potential importance of processing and storage temperatures towards overall salted butter product quality.

Several extraction techniques have been utilised for the isolation of VOC in butter to-date; such as headspace solid phase microextraction (HS-SPME) (Povolo and Contarini, 2003; Adahchour et al. 2005; Mallia et al. 2008a; O'Callaghan et al. 2016; Garvey et al. 2020; Li et al. 2020), solvent-assisted flavour evaporation (SAFE) (Mallia et al. 2009), simultaneous distillation-extraction (SDE) (Li et al. 2020), and purge & trap (PT) (Peterson and Reineccius, 2003; Povolo and Contarini, 2003). HS-SPME is the most popular technique primarily due to its ease of use, and ability to collect a wide spectrum of VOC especially when used with the triple-phase DVB/CAR/PDMS fibre (Abilleira et al. 2010). However, HS-SPME has several drawbacks relating to the limited extraction volume and a tendency to preferentially extract more volatile low molecular weight VOC (Salum et al. 2017), however this is obviously also dependent upon phase choice and conditions of extraction.

High capacity sorptive extraction (HiSorb) is a new extraction technique somewhat similar to stir bar sorptive extraction (SBSE). HiSorb with a polydimethysiloxane (PDMS) phase has been shown to be very useful in the extraction of VOC in whole milk powder in comparison to HS-SPME or thermal desorption (TD), but also for its ability to extract larger less volatile analytes, such as lactones, especially when used as a direct immersion (DI) technique rather than as a headspace (HS) technique (Cheng et al. 2021). Therefore, it appears that the VOC content of salted butter maybe better profiled using an optimized DI-HiSorb technique. This approach in association with gas chromatography olfactometry (GC-O) is potentially very suitable in identifying key VOC influencing aroma perception in salted butter,

and therefore also to determine any impacts of cow diet (pasture vrs concentrate) on the aroma perception of salted butter.

The objective of this study was to develop an optimized DI-HiSorb GCMS method to determine the impact of three distinct cow diets (outdoors on perennial ryegrass, or outdoors on perennial ryegrass/white clover, or indoors on total mixed rations) on the volatile and aromatic profile of salted butters.

6.2 Materials and Methods

6.2.1 Salted Butter Production

Raw milk was collected from three groups of Friesian cows (n=18) at the Teagasc Moorepark dairy farm, Teagasc, Animal & Grassland Research Centre, Fermoy, Co. Cork, Ireland. Each group of 18 cows were given separate diets; outdoors on perennial ryegrass (GRS), outdoors on perennial ryegrass and white clover (CLV), and indoors on a TMR as outlined in O'Callaghan et al. (2016). The animal experiment and associated procedures involving cows were approved by the Teagasc Animal Ethics Committee and authorized by the Health Products Regulatory Authority (Licence AE19132/P095 and Licence AE19132/P019), which is the competent authority in Ireland responsible for the implementation of European Union legislation (Directive 2020/63/EU) for the protection of animals used for scientific purposes. Raw whole milk (approximately 1000 kg) was collected from cows on each dietary treatment and salted butter was produced from each experimental feed system at Moorepark Technology Ltd (MTL, Moorepark, Fermoy, Co. Cork, Ireland) as outlined by O'Callaghan, et al. (2016).

6.2.2 Sample Preparation

Some preliminary work was undertaken to determine a suitable solvent and solvent volume for DI-HiSorb with salted butter in 20 ml amber head-space La-Pha-Pack extraction vials (Apex Scientific Ltd, Maynooth, Co. Kildare, Ireland). The HiSorb probe was a HiSorb-P1 inert PDMS assembly (Markes International Ltd, Bridgend, UK), which was directly immersed so that the PDMS phase remained in solution even during agitation. Fifteen ml of 15% MeOH solution (Merck, Arklow, Co. Wicklow) was found to provide sufficient salted butter solubilization without resulting in swelling of the PDMS HiSorb phase. To monitor the performance of each VOC extraction, an internal standard (IS) of 50 µL of 2-methyl-3-heptanone and 4-methyl-2-pentanol (Merck, Arklow, Co. Wicklow, Ireland) prepared at 0.001% (w/v) (1 ml in 100 ml dH2O), was added to each salted butter sample in the vial prior to extraction.

6.2.3 Optimization of Direct Immersion (DI-HiSorb) Extraction

The salted butter samples (either 0.1 g, 2.55 g, or 5 g) were carefully weighed and solubilized in 15 ml of 15% MeOH in a 20 ml amber La-Pha-Pack vials. Each HiSorb probe was pre-conditioned at 280ºC for 30 min using a U-CTE micro-chamber/thermal extractor (Markes International Ltd., Bridgend, UK) prior to use. The vials containing the sample and probe were capped and added to a HiSorb Agitator (Markes International Ltd., Bridgend, UK), and mixed at 250 rpm for preselected times and temperatures (described later). After extraction the HiSorb probes were removed from the vials and rinsed with deionized water and gently dried with a lint-free tissue prior to insertion into a clean, empty TD tube (Markes International Ltd., Bridgend, UK), which were end capped using brass long-term storage caps (Markes International Ltd., Bridgend, UK) and stored at room temperature until analysis (which occurred after no longer than 5 days).

Response Surface Methodology (RSM) was employed to optimize the extraction parameters (extraction temperature, extraction time and sample weight). Utilizing a central composite rotatable design (CCRD, $\alpha = 1$) by Design Expert version 12.0.0 (Stat-Ease, Inc., USA), the effect of extraction temperature (x1), extraction time (x2) and sample amount (x3) on the extraction of VOC from salted butter was investigated. The experimental design consisted of a 2^3-factorial design comprised of 20 experimental runs, which included 5 axial points (estimation of curvature) and 6 replicates of the centre point (estimating pure error) (Table 6.1). Twelve VOC (butanoic acid, hexanoic acid, octanoic acid, pentanal, hexanal, nonanal, ethyl hexanoate, 2,3-butanedione, 2-hexanone, 2-octanone, 2-nonanone, and δ-decalactone) were selected to evaluate the parameters based on VOC identified in previous studies (Garvey et al. 2020; O'Callaghan et al. 2016) and from preliminary work (Supplementary Table 6.1). The target compounds encompassed a range of molecular weights and chemical classes.

Data from each individual selected VOC peak response (peak area value of compound) and a combination of the peaks areas of all selected VOC were inputted into the statistical model and tested for lack of fit (ANOVA) and determination coefficient (R^2). Insignificant model terms were removed. Optimization was performed to obtain the maximum extraction efficiency i.e. identifying the extraction parameters that result in the greatest area value for each selected VOC and total peak area.

Table 6.1 Central composite rotatable design (20) showing the levels of each independent variables (temperature (x1, ^{o}C), extraction time (x2, h), amount of sample (x3, g)) constructed using Design Expert (Stat-Ease, Inc., USA) for the DI-HiSorb volatile extraction of salted butter.

Run	X_1:Extraction temperature (^{o}C)	X_2:Extraction time (h)	X_3:Sample amount (g)
1	40	1	0.1
2	40	24	0.1
3	40	1	5
4	40	24	5
5	40	12.5	2.55
6	47.5	1	2.55
7	47.5	24	2.55
8	47.5	12.5	0.1
9	47.5	12.5	5
10	47.5	12.5	2.55
11	47.5	12.5	2.55
12	47.5	12.5	2.55
13	47.5	12.5	2.55
14	47.5	12.5	2.55
15	47.5	12.5	2.55
16	55	1	0.1
17	55	24	0.1
18	55	1	5
19	55	24	5
20	55	12.5	2.55

Supplementary Table 6.1 Selected volatile compounds in salted butter for optimization of DI-HiSorb parameters

Chemical class	Volatile compound	Kovats index [a]	Sensory description [b]
Acid	Butanoic acid	805	Rancid cheesy, putrid, sweaty
	Hexanoic acid	990	Doughy, pungent, blue cheese, sour, sweaty
	Octanoic acid	1180	Goaty, waxy, soapy, musty, rancid, cheesy
Aldehyde	Pentanal	697	Pungent, almond like, chemical, malty,apple
	Hexanal	801	Green, slightly fruity, lemon, herbal
	Nonanal	1106	Mushroom-like, floral, green, waxy, fatty
Ester	Ethyl hexanoate	925	Acidic, sweaty, cheesy, sharp, goaty
Ketone	2,3-Butanedione	596	Buttery
	2-Hexanone	790	Earthy, strong fruity, wood pulp, hay
	2-Octanone	992	nice fruity, blue and parmesan cheese-like
	2-Nonanone	1094	Malty, fruity, hot milk, smoked cheese
Lactone	δ-Decalactone	1506	Waxy, sweet,Coconut like, peach

a relevant reference linear retention indices

b Odor descriptors www.goodscentcompany.com

6.2.4 Volatile Analysis by GC-MS

Desorption of the HiSorb probes was automated by a Unity 2 Thermal Desorption unit (Markes International Ltd., Bridgend, UK), with a Series 2 Ultra TD autosampler (Markes International Ltd., Bridgend, UK) to concentrate the VOC and remove moisture prior to direct transfer to the GC-MS. The HiSorb probes were dry purged for 2 min using a 1:20 split. The first stage of desorption was carried out at 150°C for 5 min, and the second stage was performed at 300°C for 5 min. Final tube desorption was performed using a 1:10 split. The cold trap used was a material emissions focusing trap (U-T12ME-2S, Markes International Ltd, Bridgend, UK) maintained at 30°C with a 50 ml/min gas flow during tube desorption. Prior to trap desorption, a 2 min pre-trap fire purge with a 1:50 split was performed. Trap desorption was performed at 30-300°C at a rate of 24°C/min and held for 5 min with 1:10 split.

The GC-MS system was an Agilent 7890A GC with an Agilent 5977B MS detector (Agilent Technologies Ltd., Cork, Ireland). The column used was a capillary DB-624 UI (60 m x 0.3 mm x 1.8 µm) (Agilent Technologies Ltd., Ireland) with helium as the carrier gas held at a constant flow of 1 ml/min. The column temperature started at 40°C, then held for 5 min, increased to 230°C at 5°C/min and held at 230°C for 35 min. The total run time was 78 min. Injector temperature was set at 250°C and the transfer line was set at 150°C. The mass spectra of VOC were generated by a MS quadrupole detector with ionization voltage of 70 eV, 3.32 scans/s and a scanning mass range of 35-350 amu. The ion source temperature was 220°C and the interface temperature was set at 280°C. Autotunes were carried out weekly.

Volatiles were identified using mass spectra comparisons to the NIST 2014 mass spectral library, an in-house library created using authentic compounds with target and qualifier ions and linear retention indices for each compound using the Kovats index (van Den Dool and Kratz, 1963). Spectral deconvolution was also performed to confirm identification of compounds using AMDIS. Batch processing of the samples was carried out using metaMS (Wehrens et al. 2014).

6.2.5 Model Validation

Validation of the model was performed by applying the optimized DI-HiSorb conditions to the salted butter matrix analyzed in triplicate, and by comparing the average response values obtained to the values predicted by the regression model.

6.2.6 Application of the Optimized DI-HiSorb Extraction Method

Once the DI-HiSorb extraction method was optimized, it was applied to three different salted butter samples generated from three cow diets (GRS, CLV and TMR) in triplicate.

6.2.7 Identification of Odor Active Volatiles by GC-O

DI-HiSorb-GC-O analysis was performed on an Agilent 7890 GC with a flame ionization detector, 5973N MS detector (Agilent Technologies, Ltd, Cork, Ireland), and a Gerstel ODP-3 olfactory sniffing port (Anatune Ltd, Cambridge, UK). The volatile compounds were separated on DB-624 UI (20 m × 1.8 mm × 1 μm) (Agilent Technologies Ltd, Ireland) column. Helium was used as the carrier gas with a constant flow of 1.209 ml/min and held at a pressure of 9.8 psi. Eluting compounds were split 1:1:1 into the MS detector, flame ionization detector and the sniffing port simultaneously by means of a column flow splitter. The optimized extraction procedures were applied to salted butter samples (section 6.3). Instrument conditions

for analysis was the same as described in section 6.4 with the following exceptions. The column oven was set at 40°C for 2 min, increased to 140 °C at a rate of 6°C /min, then increased to 220°C at a rate of 15°C /min, held for 5min, then increased to 260 °C at a rate of 15 °C /min and held for 5 min for a total run time of 36.67 min. The total runtime was shortened to reduce the risk of assessors experiencing fatigue during a sniffing session. Trap desorption was performed using splitless conditions to maximize responses for the assessors. In addition, the transfer line to the sniffing port was conditioned with humidified air to reduce olfactory fatigue and prevent the occurrence of condensation droplets collecting in the nasal port.

Five experienced panelists conducted GC-O analysis of each salted butter samples in triplicate. These panelists undertook a number of additional GC-O analysis sessions prior to evaluating the samples formally, in order to familiarize themselves with the samples and to create a standardized check list of aroma descriptors with approximate retention times. Each session was ~30 min and each assessor carried out only two sessions per day. The panelists were also asked to rate the intensity of the eluted aroma using a four-point category scale (1 = weak, hardly recognizable odor; 2 = clear but not intense odor; 3 = intense odor; 4 = very intense odor), recorded by a Gerstel OID Interface/ODP-Recorder (Anatune Ltd, Cambridge, UK) similar to that used by Vilar et al. (2021). Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, comparison of LRI to the mid polar column from the previous analysis and with standards where possible. Spectral de-convolution was also performed to confirm identification of compounds using AMDIS.

6.2.8 Data Analysis

The RSM design and desirability function were accomplished with the aid of Design Expert Version 12 (Stat-Ease Inc. Minneapolis, MN). One-way ANOVA was used to determine if the model itself was significant and capable of being a beneficial predictive model. Principal component analysis (PCA) biplots of the volatile data were carried out to aid the visual association of the selected VOC using the "factoextra" and "FactoMineR" packages within R (v 3.4.1, R Foundation for Statistical Computing, Vienna, Austria). To classify salted butter samples in a supervised multivariate model, partial least squares discriminant analysis (PLS-DA) was performed. The Variable Importance Plot (VIP) scores summarized the individual X variables and have an influence on the PLS model and rank in terms of importance on the Y axis (with variables of the highest importance at the top). Metabolic Analyst 4.0 (McGill University, Montreal, QC, Canada) software was used to perform the Variable importance plot (VIP) in PLS-DA (Chong and Xia, 2020).

6.3 Results and Discussion

6.3.1 Experimental Design for Optimization of DI-HiSorb Parameters

The CCRD experimental results demonstrated the main interaction and quadratic effect of the extraction parameters on the VOC response (Table 6.2) using analysis of variance (ANOVA). The final response surface-reduced quadratic model were satisfactory ($p < 0.0001$) in explaining the variability of responses amongst the selected 12 VOC, with satisfactory determination coefficients (R^2) ranging from 0.8366 (ethyl hexanoate) to 0.9388 (nonanal). This demonstrated good correlation between the experimental data and the fitted model. The satisfactory determination coefficients (total area of 12 selected VOC) for 0.9109, demonstrated that this model can explain up to 91.1% of the variability of the responses. The lack of fit for total area of all selected VOC was not significant ($p > 0.05$) indicating the data fitted the

regression model adequately. The optimized conditions were determined as an extraction time of 24 hr at 40°C and 0.1 g of sample based on the desirability factor using Design Expert software. The optimized DI-HiSorb technique was developed using Design Expert version 12 ‘desirability function’, which combines the optimum conditions for each response (volatile chemical) to identify a method that achieves the highest desirability between 0 and 1 (ideally closer to 1). The optimization desirability value of this proposed method was 0.997 (Supplementary Table 6.2). To validate the proposed DI-HiSorb model, these optimized extraction conditions: were subsequently applied to the same salted butter sample and analysed in triplicate. The verification of the experimental results are listed in Table 6.3 and the experimental total peak area of target selected VOCs was not significantly different from the predicted values (p 0.87), demonstrating the effectiveness of the extraction procedure conditions.

Extraction time is a very important parameter as sufficient time is needed for all VOC to reach an equilibrium on the sorbent (PDMS). As the capacity of HiSorb probes is greater than a SPME fibre, it reduces problems associated with VOC sorbent phase affinity/displacement, which is a known limitation of SPME fibres (Cheng et al. 2022). Extraction time applies to both very volatile and heavier/semi-volatile compounds, all of which take different times to interact with the sorbent (Prosen and Zupančič-Kralj, 1999). Extraction time (B) had a statistically positive effect on VOC extraction in the model. The response of all 12 selected VOC varied between $p < 0.0001$ and p 0.044 (Table 6.2). Thus, increasing extraction time improved the efficiency of extraction. Similar results were found by Dadali and Elmaci, (2019) for margarine. Extraction temperature was also determined to have a statistically important positive effect on the recovery of all 12 selected VOC, except for 2,3-butanedione (diacetyl) (p 0.4598, Table 6.2). This is likely because diacetyl had the

lowest boiling point of the selected VOC and is very volatile with a vapour pressure of 56.8 mm Hg at 25°C (Clarke and Winter, 2015), thus increasing temperature is more likely to impact on its volatility, especially in a hydrophobic matrix such as salted butter. In solid food matrices with high fat contents, such as salted butter the fat phase acts as a solvent for many VOC making extraction more difficult. In general, heat provides energy for analyte molecules to overcome barriers within the sample matrix, thus facilitating release of more analytes into the sorbent phase. The optimum extraction temperature was determined as 40°C, which was previously used to extract VOC in milk by SBSE (Wherry et al. 2019) and whole milk powder by HiSorb (Cheng et al. 2021) and unlikely to result in artefact formation due to thermal degradation.

In relation to sample amount, 0.1g was found to be optimum (Supplementary Table 6.2). This amount (0.1 g) was found to only have a significant effect on the abundance of hexanoic acid ($p < 0.05$), pentanal ($p < 0.05$) and ethyl hexanoate ($p < 0.05$). The effectiveness of DI-HiSorb in relation to VOC extraction is based on an equilibrium amongst the sorbent phase (PDMS), the solvent (15% MeOH), and the sample (salted butter) amount, where the sample amount directly affects both the solvent and sample (Vas and Vekey, 2004). Previous work by Penton (1999) demonstrated that extraction efficiency and sensitivity was enhanced for non-polar samples when the ratio of liquid phase increased in relation to the sample amount. However, once sorbent adsorption reaches an equilibrium no more changes can occur.

The interaction effect of extraction temperature and extraction time for 0.1 g of sample was significant ($p < 0.05$) for 6 (hexanoic acid, pentanal, hexanal, nonanal, ethyl hexanote and δ-decalactone) of the 12 selected VOC (Table 6.2), where abundance increased as extraction temperature decreased and extraction time increased concurrently (Supplementary Figure 6.1a). Additionally, the interaction

between sample amount and extraction temperature only impacted the recovery of diacetyl, where abundance increased as extraction temperature increased and sample amount decreased concurrently (p 0.0068, Table 6.2, Supplementary Figure 6.1a) (as previously mentioned increasing temperature is more likely to impact on the volatility of diacetyl in a hydrophobic matrix such as salted butter). Moreover, interaction between extraction time and sample amount influenced the abundance of 2-hexanone, 2-octanone and 2-nonanone ($p < 0.05$) as their peak areas increased with increased extraction times and decreased sample amounts (Supplementary Figure 6.1a). Overall, for the total area of all 12 selected VOC longer extraction times and smaller sample amounts resulted in greater abundance (Supplementary Figure 6.1b). However, the interaction effects of extraction temperature and extraction time were not significant (p 0.0669) for the total area of all 12 selected VOC (Table 6.2).

Table 6.2 ANOVA results for each response variables of optimization process

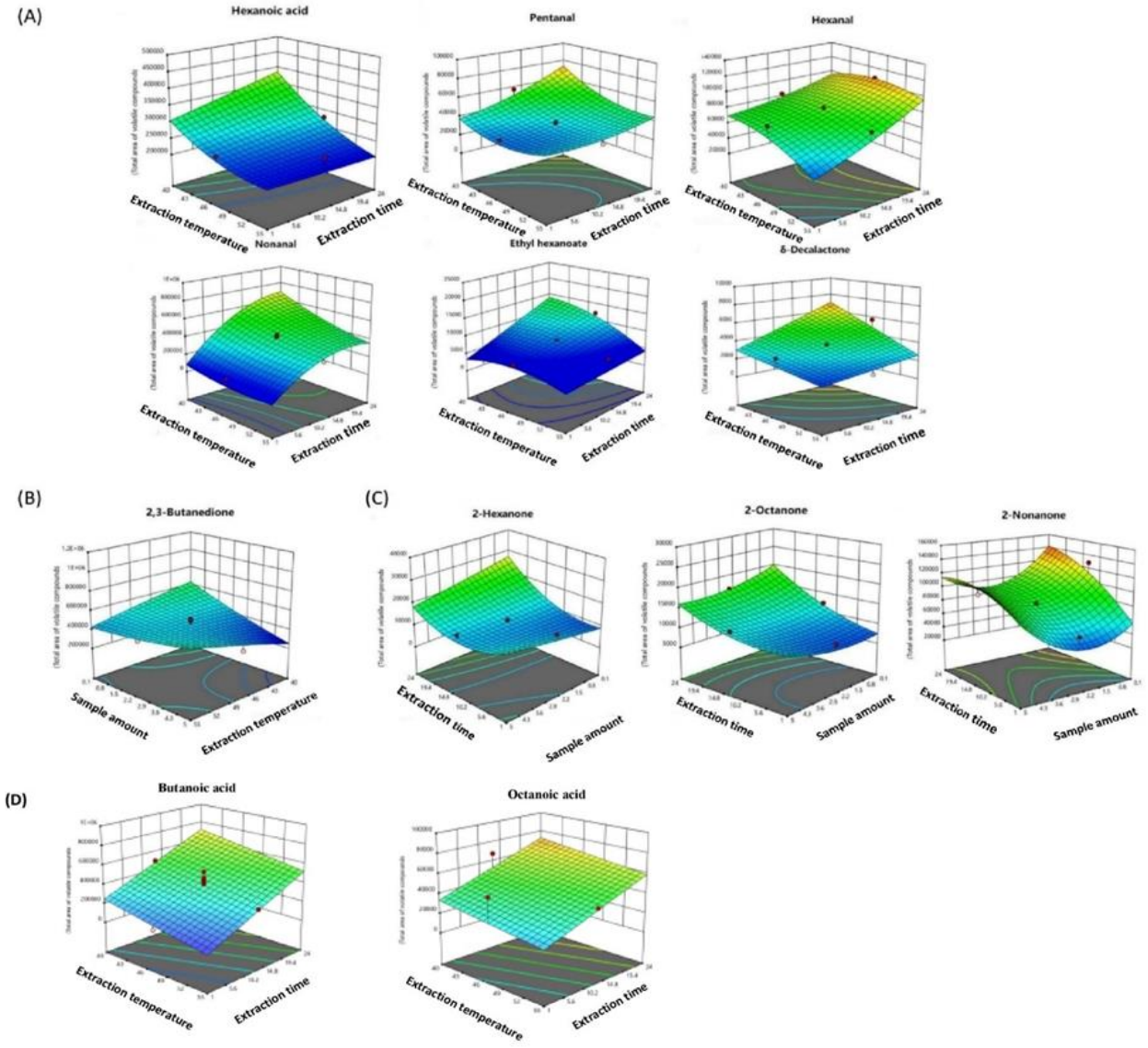
Target Volatile Compound	Butanoic acid p value	Hexanoic acid p value	Octanoic acid p value	Pentanal p value	Hexanal p value	Nonanal p value	Ethyl hexanoate p value	2,3-Butanedione p value	2-Hexanone p value	2-Octanone p value	2-Nonanone p value	δ-Decalactone p value	Total area of 12 selected volatile compounds p value
Model	0.0006	0.0002	0.0049	0.0045	0.0007	< 0.0001	0.0060	0.0017	0.0019	0.0014	< 0.0001	< 0.0001	< 0.0001
A-Extraction temperature	0.0088	< 0.0001	0.0002	0.0079	0.0276	0.0014	0.0094	0.4598	0.0228	0.0023	0.0006	< 0.0001	< 0.0001
B-Extraction time	< 0.0001	0.0385	0.0440	0.0008	< 0.0001	< 0.0001	0.0038	0.0001	0.0003	0.0001	< 0.0001	0.0002	< 0.0001
C-Sample amount	0.4274	0.0247	0.4636	0.0459	0.0532	0.2169	0.0313	0.0992	0.6310	0.7580	0.9849	0.1014	0.7233
AB	0.4384	0.0279	0.9522	0.0365	0.0474	0.0234	0.0445	0.8964	0.2085	0.1390	0.5410	0.0218	0.0669
AC	0.1037	0.2452	0.8962	0.3433	0.8899	0.3723	0.4615	0.0068	0.0744	0.1057	0.5859	0.0563	0.2282
BC	0.1034	0.3194	0.9016	0.4268	0.1448	0.1496	0.1753	0.2406	0.0038	0.0483	0.0040	0.7031	0.0073
Lack of Fit	0.0832	0.7234	0.5764	0.6643	0.7139	0.7668	0.9432	0.5876	0.4739	0.2280	0.4312	0.7689	0.1295
R^2	0.9019	0.9210	0.8435	0.8469	0.8992	0.9388	0.8366	0.8767	0.8735	0.8813	0.9361	0.8524	0.9109

Table 6.3 Results of statistical analysis for verification of optimization

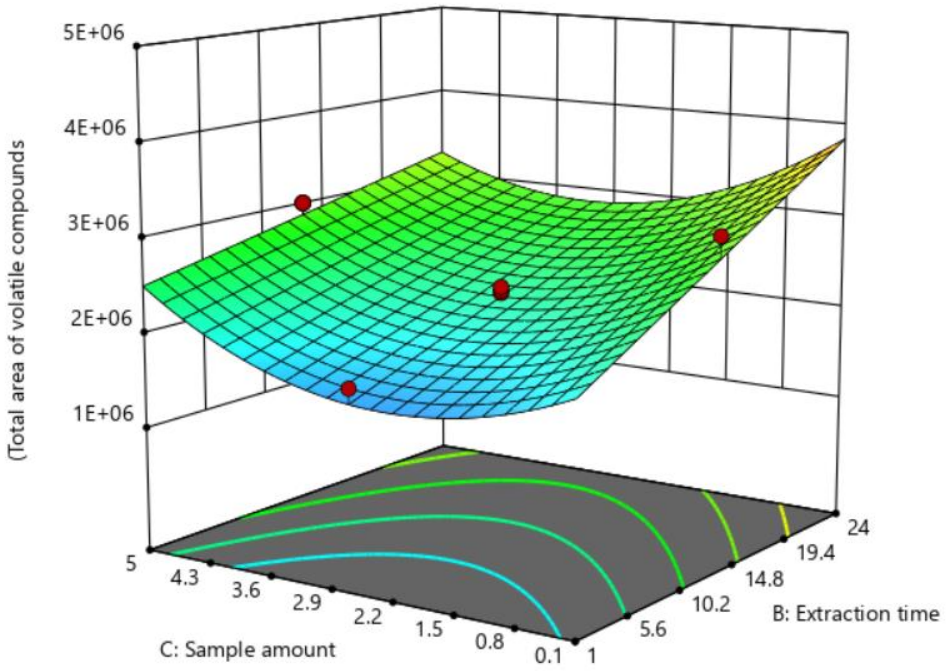
Reponses	Predicted value	Experimental value (average)	difference	F	P-value
Total area of volatile compounds	4314002.00	4324765.67	10763.67	0.03	0.87

Supplementary Table 6.2 The optimum conditions for 12 selected volatile compounds

	Extraction temperature (°C)	Extraction time (h)	Sample amount (g)	Total area of volatile compounds	Desirability
Butanoic acid	40	1	0.1	698297	0.895
Hexanoic acid	40	24	0.1	222247	0.719
Octanoic acid	40	1	2.6	52507	0.973
Pentanal	40	24	0.1	77707	0.901
Hexanal	47	24	0.1	126082	1
Nonanal	40	24	0.1	750562	0.794
Ethyl hexanote	40	24	0.1	11983	0.897
2,3-Butanedione	44.8	7	2.6	446118	1
2-Hexanone	49.7	24	0.1	22702	0.874
2-Octanone	40	24	0.1	18676	0.882
2-Nonanone	40	24	0.1	138748	0.903
δ-Decalactone	40	24	0.1	4028	0.738
Total area for all 12 selected VOC	40	24	0.1	4314002	0.997


(A)
Hexanoic acid
Extraction temperature
Extraction time
Nonanal
Pentanal
Extraction temperature
Extraction time
Ethyl hexanoate
Hexanal
Extraction temperature
Extraction time
δ-Decalactone
Extraction temperature
Extraction time
Extraction temperature
Extraction time
Extraction temperature
Extraction time
(B)
2,3-Butanedione
Sample amount
Extraction temperature
(C)
2-Hexanone
Extraction time
Sample amount
2-Octanone
Extraction time
Sample amount
2-Nonanone
Extraction time
Sample amount
(D)
Butanoic acid
Extraction temperature
Extraction time
Octanoic acid
Extraction temperature
Extraction time

Supplementary Figure 6.1a Response Surface graphs for the dependent variable (A) total area value vs. extraction time (h) and extraction temperature (°C); (B) total area value vs. extraction temperature (°C) and sample amount (g); (C) total area value vs. extraction time (h) and sample amount (g) displaying the optimum extraction conditions for the target compounds from salted butter sample. (D) No significant interaction effect between factors ($P > 0.05$)



Supplementary Figure 6.1b: Response Surface graphs for the dependent variable (total area value) vs. extraction time (h) and sample amount (g) showing the optimum extraction conditions for the total area response for all 12 selected VOC in the salted butter sample.

6.3.2 Volatile Compounds in Salted Butter

The optimized DI-HiSorb GCMS method was applied to salted butter samples produced from milk derived from the 3 diets; GRS, CLV and TMR. In total, 66 VOC were identified (Table 6.4), consisting of 19 aldehydes, 13 ketones, 6 esters, 9 acids, 5 lactones, 6 benzenes, 4 alcohols, 2 furans, 1 terpene and 1 sulfur compound. This was considerably more VOC extracted than in other studies. For example HS-SPME studies have identified 30 VOC in salted butter (Garvey et al. 2020); 22 VOC in unsalted butter by HS-SPME (Li et al. 2020), 21 VOC in butter (Povolo and Contarini, 2003), whilst 23 VOC were identified in unsalted butter by SDE (Li et al. 2020), and 43 VOC in butter by PT (Povolo and Contarini, 2003). The major volatile classes in these 3 salted butter samples were aldehydes, ketones, acids, esters, benzene/phenols and lactones. The abundances of 40 of the 66 VOC varied significantly ($p < 0.05$) based on cow diet (GRS, CLV and TMR) (Table 4); 13 aldehydes, 7 ketones, 6 acids, 5 esters, 5 lactones, 1 alcohol, 1 terpene, 1 furan and 1 phenolic compound. These results clearly demonstrate the impact of cow diet on the volatile profile of salted butter.

A significant number of aldehydes can be transferred from diet into milk and butter (Glover et al., 2012; Garvey et al., 2020; Clarke et al., 2022), but most are likely to be primarily derived from oxidation of PUFA generated during butter production and over storage (Peterson and Reineccius, 2003). The abundance of 13 aldehydes (acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, (E)-2-nonenal, (E)-2-decenal, 2-undecanal, dodecanal, and tetradecanal) were significantly ($P < 0.05$) influenced by diet. Butanal, nonanal, dodecanal and tetradecanal are all products of lipid oxidation and were significantly ($P < 0.05$) higher in GRS salted butter. Both butanal (Povolo, et al., 2003) and nonanal (Garvey et al., 2020) were

previously identified in butter with nonanal identified as an important odor active compound in sweet cream butter (Peterson and Reineccius, 2003) and salted butter (Lozano, et al., 2007). However neither dodecanal or tetradecanal have been previously identified in butter. Propanal, pentanal, hexanal, heptanal, octanal, (E)-2-decanal and 2-undecanal were all significantly ($P < 0.05$) more abundant in CLV and GRS salted butter and each are also products of lipid oxidation. Pentanal has been previously identified in butter (Christensen and Hølmer, 1996; Mallia et al., 2008b; Mallia et al 2009; O'Callaghan et al., 2016; Garvey et al., 2020), and hexanal as an important odor active VOC in in salted butter (Lozana et al., 2007) and in sweet cream butter (Mailla et al., 2008a). Heptanal has also been widely identified in butter (Christensen and Hølmer, 1996; Povolo et al., 2003: Mallia et al., 2008b; Mallia et al., 2009; O'Callaghan et al., 2016; Garvey et al., 2020), as has octanal (Christensen and Hølmer, 1996; Povolo et al., 2003; Mallia et al 2009). Povolo and Contarini (2003) identified 2-decanal in butter, but not did not specifically identify (E)-2-decanal. Neither 2-undecanal or propanal have been identified in butter previously. (E)-2-Nonenal was significantly ($P < 0.05$) more abundant in GRS salted butter and has been previously identified as a key odour active VOC in sweet cream salted butter (Peterson and Reineccius, 2003), Irish sour cream butter (Schieberle et al., 1993), and butter (Lozano et al., 2007; Mallia et al., 2008a; Mallia et al., 2009). Acetaldehyde was also significantly more abundant ($P<0.05$) in the GRS salted butter, and is a product of pyruvate metabolism, and has previously been identified as aroma active in sweet cream butter (Petererson and Reineccius, 2003). It is noteworthy that salted butter derived from pasture based feeding systems (CLV and GRS) contained significantly more aldehydes resulting from lipid oxidation than salted butter produced from TMR diets, similar to that found by Garvey et al. (2020). This is likely a direct result of the

established impact of diet on the fatty acid profile of milk (O'Callaghan et al., 2019; Kalač and Samková, 2010), but may also be linked to direct transfer of aldehydes from the diet (Clarke et al., 2022).Lactones are cyclic compounds, derived from the intramolecular esterification of hydroxyacids (Urbach, 1997), which contribute to sweet, creamy and fatty flavors in butter (Sarrazin et al., 2011). The DI-HiSorb method has proven to be very effective in extracting lactones. All 5 lactones identified in this study were statistically impacted by diet and were the only classes of volatiles whose presence or absence was impacted by diet rather than just abundances. γ-Octalactone, γ-6-(Z)-dodecenolactone ((Z)-dairy lactone) and γ-dodecalactone were only present in the CLV salted butter sample. γ-Octalactone has also been identified previously in butter (Christensen & Hølmer, 1996; Mallia et al., 2009), and as odor active in Irish sour cream butter (Schieberle et al., 1993). (Z)-Dairy lactone was found to be odor active in sweet cream butter (Mallia et al., 2009), unsalted butter (Li et al., 2020) and in Irish sour cream butter (Schieberle et al., 1993). γ-Dodecalactone was also found to be odor active in unsalted butter (Li et al,. 2020) and in sweet cream butter (Peterson and Reineccius, 2003; Mallia et al., 2008a). γ-Dodecalactone was the most abundant lactone in these salted butter samples. δ-Dodecalactone was present all 3 salted butter samples but significantly ($P < 0.05$) more abundant t in the CLV salted butter samples, and has been previously identified as an important odor active VOC in butter (Lozano et al., 2007; Mallia et al 2009; Li et al., 2020). δ-Decalactone was also present in all 3 butter samples, but statistically highest ($P < 0.05$) in GRS and TMR salted butter. Multiple studies have identified δ-decalactone as odor active and thus an important VOC in butter (Schieberle et al., 1993; Lozano et a., 2007; Mallia et al., 2008; Mallia et al., 2009; Garvey et al., 2020; Li et al., 2020). Overall most of the lactones were more prevalent in the CLV salted butter samples, this was not the case in the study by

Garvey et al. (2020) who did not find any impact of diet on lactone formation in similar butters. However this may be due to the different volatile extraction methods used, as HS-SPME is quite poor in extracting lactones as opposed to DI-HiSorb (Cheng et al., 2021). As lactones are derived from fatty acids by hydroxylation in the rumen then created by cycles of β-oxidation and lactonization (Saeki et al., 2022; Zia et al., 2022), this also further highlights the importance of the impact of cow diet on their formation in milk and subsequent dairy products.

The abundance of 7 ketones were also significantly ($P < 0.05$) influenced by diet. The most abundant ketone in these salted butter samples which varied by diet was diacetyl, which was significantly ($P < 0.05$) higher in the CLV salted butter. Diacetyl is a very odour-active VOC derived from pyruvate metabolism, however it has also recently been shown that it may also be transferred directly from the diet and higher in the TMR feed (Clarke et al., 2022). Previous studies have identified diacety as odor active in butter (Mallia et al., 2008b), sweet cream butter (Peterson and Reineccius, 2003; Lozano et al., 2007; Mallia et al., 2008a) and in Irish sour cream butter (Schieberle et al., 1993). It is worth nothing that as previously stated the recovery of diacetyl was impacted by extraction temperature, extraction time and sample amount. A number of methyl ketones were also significantly ($P < 0.05$) impacted by diet (2-pentadecanone, 2-nonanone, 2-octanone, 2-hexanone, 3-pentanone and 2-undecanone). These methyl ketones are derived from their corresponding aldehyde via the oxidation of PUFA (La Terra et al., 2010) and therefore also linked to the dietary impact on milk fatty acid content. In this case 2-pentadecanone, 2-undecanone and 3-pentanone were significantly higher ($P < 0.05$) in TMR salted butter. 2-Pentadecanone, although not commonly found in butter but was identified by Li et al., (2020) in unsalted butter and has previously been linked to

thermally derived off-flavors in milk (Vazquez-Landaverde et al., 2005). 2-Undecanone was found to be odor active in butter by Mallia et al., (2008b), and 3-pentanone is a product of pyruvate metabolism and has not previously been found in butter. Both 2-octanone and 2-nonanone were statistically more abundant in CLV and GRS salted butter. 2-Octanone was identified in butter by Povolo and Contarini, (2003). 2-Nonanone, was identified in butter multiple times (Christensen and Hølmer, 1996; Povolo and Contarini, 2003; O'Callaghan et al., 2016; Garvey et al., 2020) and as odor active in butter (Mallia et al., 2008b; Mallia et al., 2009). 2-Hexanone was statistically ($P < 0.05$) more abundant in GRS salted butter and has been previously identified in butter (Povolo and Contarini, 2003), GRS feed (Clarke et al., 2022) and unsalted butter (Li et al., 2020).

The abundance of 6 short chain fatty acids were also significantly ($P < 0.05$) influenced by diet. These are derived from a range of potential sources; lipolysis, *de novo* synthesis, carbohydrate metabolism, directly transferred from the diet, produced by lipolytic activity from lipoprotein lipase or by esterases from psychrotrophic bacteria (O'Callaghan et al., 2016; Palmquist et al., 1993; Clarke et al., 2022). Butanoic acid was by far the most abundant acid and was significantly higher ($P < 0.05$) in CLV salted butter, which was different to a study by O'Callaghan et al. (2016) and Garvey et al. (2020) on similar salted butter samples. Butanoic acid has previously been identified as a key odor active VOC in butter (Mallia et al., 2009; Lozano et al., 2007), unsalted butter (Li et al., 2020), Irish sour cream butter (Schieberle et al., 1993), and sweet cream butter (Peterson and Reineccius, 2003: Mallia et al., 2008a). Heptanoic acid was also significantly ($P < 0.05$) more abundant in CLV salted butter, has not been previously identified in butter. Nonanoic acid was statistically ($P < 0.05$) most abundant of CLV and GRS salted butter. Garvey et al. (2020) also found that

nonanoic acid was more abundant higher in CLV salted butter, but in general it has not been readily identified in butter. Hexanoic acid, decanoic and valeric acid were statistically ($P < 0.05$) more abundant in TMR salted butter. Hexanoic acid has already been identified as an important aroma active VOC in butter (Mallia et al., 2008b), unsalted butter (Li et al., 2020), Irish sour cream butter (Schieberle et al., 1993) and in sweet cream butter (Peterson and Reineccius, 2003; Mallia et al., 2008a). Decanoic acid was identified in unsalted butter by Li et al (2020) but not found to be odor active. Clarke et al. (2022) also found more decanoic acid in milk from a TMR diet than either GRS or CLV. Valeric acid (pentanoic acid) was the least abundant acid in these butter samples and has not previously been identified in butter. It is likely a product of carbohydrate metabolism and/ or *de novo* synthesis.The abundance of five esters were also significantly influenced by diet. Ester content is dependent upon the abundance of primary alcohols and carboxylic acids. Methyl hexanoate, methyl dodecanoate and methyl butanoate were all significantly higher ($P < 0.05$) in TMR salted butter. The higher levels of methyl esters in salted butter derived from TMR may be due to additional methanol in milk from the TMR diet as it contains more carbohydrates than the either pasture diet (Kilcawley et al., 2018). Both methyl hexanoate and methyl butanoate were identified as odor active in in sweet cream butter (Mallia et al., 2008b), but methyl dodecanoate has not previously been identified in butter. Ethyl butanoate was significantly ($P < 0.05$) more abundant in CLV salted butter and was previously identified as odor active in butter by Lozano et al. (2007). Ethyl hexanoate was significantly ($P < 0.05$) more abundant in GRS salted butter and has also been previously identified as odor active in butter (Mallia et al., 2009).

The abundance of only one alcohol (2-ethyl-1-hexanol) was significantly influenced by diet. 2-Ethyl-1-hexanol was significantly more abundant ($P < 0.05$) in

the GRS salted butter, but has not been identified in butter previously. D-Limonene was the only terpene found in these salted butter samples but was significantly higher ($P < 0.05$) in CLV salted butter. It has previously been identified in butter (Christensen and Hølmer, 1996; Povolo and Contarini, 2003) and in unsalted butter (Li et al., 2020), but only as odor active sweet cream butter (Mallia et al., 2008b). The only furan identified in these salted butter samples was 2-pentylfuran which was significantly more abundant ($P < 0.05$) in GRS salted butter. 2-Pentyl furan is formed from lactose through the Maillard reaction (Li and Wang, 2016), or also by lipid oxidation (Delgado et al., 2015), but has not previously been identified in butter. The only benzene/phenolic VOC impacted by diet was phenol, which was significantly higher ($P < 0.05$) in CLV and GRS salted butters, similar to that found in cheeses and raw milk from pasture (Bovolenta et al., 2014; Clarke et al., 2022). Li et al (2020) identified phenol in unsalted butter but found that it was not odor active.

Figure 6.1 is a Variable Importance in Projection (VIP) plot displaying the VOC with a VIP >1 which are the most significant VOC responsible for the differentiation between these salted butter samples based on (CLV, GRS and TMR) diet. It is very interesting to note the most discriminatory VOC are 3 lactones, all with a VIP score > 2; γ-octalactone, (Z)-dairy lactone and γ-dodecalactone all highly associated with CLV salted butter. From the 20 most discriminatory VOC 14 (γ-octalactone, dairy lactone, γ-dodecalactone, d-limonene, 2-undecanal, heptanoic acid, butanal, hexanal, 2-(E)-decenal, dodecanal, octanal, tetradecanal, ethyl butanoate and nonanal) were highly associated with CLV salted butter, highlighting a very distinct impact on the volatile profile of this butter in comparison to the GRS and TMR salted butters. Only 3 VOC with VIP scores > 1 were mostly associated with TMR salted

butter (pentanoic acid, decanoic acid and methyl hexanoate) or with GRS salted butter (propanal, 2-octanone and 2-hexanone).

Figure 6.2 is a principal component analysis (PCA) plot highlighting the association of the 66 VOC with the CLV, GRS and TMR salted butters. It is very apparent from Figure 2 that the volatile profile of each salted butter sample is clearly distinct from each other based on cow diet, with very good repetition within the replicates of each diet (due to the close proximity of each replicate). This study has shown considerable VOC diversity between samples based on diet, but that salted butter derived from GRS and CLV are somewhat more similar than salted butter derived from the TMR diet. This work has also strengthened the significant impact of VOC directly and indirectly derived from with fatty acids as impacted by diet.

Table 6.4 Volatile compounds identified in salted butter samples.

		Retention Index		Identification	Feeding System		
Volatile Compounds	CAS number	RI	REF RI	Methods	CLV	GRS	TMR
			Acids				
Acetic acid	64-19-7	685	662 [a]	MS, RI, STD	0.3	0.36	0.29
Propanoic acid	79-09-4	778	786 [a]	MS, RI	0.05	0.06	0.07
Butanoic acid	107-92-6	861	882 [a]	MS, RI, STD	1.29^{x}	1.00^{y}	0.94^{y}
Valeric acid	109-52-4	959	973 [a]	MS, RI, STD	0.02^{y}	0.02^{y}	0.06^{x}
Hexanoic acid	142-62-1	1050	1069 [a]	MS, RI, STD	0.71^{z}	0.56^{y}	1.11^{x}
Heptanoic acid	111-14-8	1153	1164 [a]	MS, RI, STD	0.17^{x}	0.12^{y}	0.10^{y}
Octanoic acid	124-07-2	1242	1261 [a]	MS, RI, STD	0.22	0.29	0.38
Nonanoic acid	112-05-0	1343	1353 [a]	MS, RI	0.42^{x}	0.42^{x}	0.33^{y}
Decanoic acid	334-48-5	1441	1452 [a]	MS, RI, STD	0.30^{z}	0.50^{y}	0.69^{x}
			Alcohols				
Isopropyl Alcohol	67-63-0	540	539 [d]	MS, RI, STD	0.19	0.24	0.16
1-Hexanol	111-27-3	915	917 [a]	MS, RI, STD	0.04	0.06	0.05
1-Hexanol,2-ethyl-	104-76-7	1090	1076 [e]	MS, RI, STD	0.15^{y}	0.33^{x}	0.13^{y}
1-Octanol	111-87-5	1129	1120 [a]	MS, RI, STD	0.07	0.09	0.07
			Aldehydes				
Acetaldehyde	75-07-0	437	449 [a]	MS, RI	0.18^{y}	0.27^{x}	0.16^{z}
2-Propenal	107-02-8	522		MS	0.4	0.32	0.35
Propanal	123-38-6	526	523	MS, RI	0.16^{x}	0.18^{x}	0.09^{y}
Butanal	123-72-8	627	622 [c]	MS, RI, STD	0.43^{x}	0.34^{y}	0.26^{y}
Pentanal	110-62-3	734	733 [b]	MS, RI, STD	0.35^{x}	0.44^{x}	0.27^{y}
Hexanal	66-25-1	840	828 [a]	MS, RI, STD	0.65^{x}	0.69^{x}	0.40^{y}
2-Hexenal,(E)-	6728-26-3	908	901 [a]	MS, RI	0.02	0.03	0.02
Heptanal	111-71-7	943	938 [a]	MS, RI, STD	0.44^{x}	0.42^{x}	0.35^{y}
Benzaldehyde	100-52-7	1037	1019 [a]	MS, RI, STD	0.17	0.23	0.14

Continued Table 6.4

Octanal	124-13-0	1059	1043 [a]	MS, RI, STD	0.43[x]	0.46[x]	0.30[y]
2,4-Heptadienal,(E,E)-	4313-03-5	1080	1074 [b]	MS, RI, STD	0.06	0.06	0.05
Nonanal	124-19-6	1160	1147 [a]	MS, RI, STD	2.29[x]	2.10[y]	1.74[y]
2-Nonenal,(E)-	18829-56-6	1224	1218 [b]	MS, RI, STD	0.08[y]	0.12[x]	0.06[y]
Decanal	112-31-2	1253	1252 [a]	MS, RI, STD	0.32	0.3	0.24
2-Decenal, (E)-	3913-81-3	1330	1327 [c]	MS, RI	0.12[x]	0.11[x]	0.08[y]
Undecanal	112-44-7	1357	1354 [d]	MS, RI, STD	0.03	0.04	0.05
2-Undecenal	2463-77-6	1436		MS	0.11[x]	0.12[x]	0.06[y]
Dodecanal	112-54-9	1459		MS	0.22[x]	0.17[y]	0.15[y]
Tetradecanal	124-25-4	1663		MS	0.25[x]	0.21[y]	0.18[y]
			Esters				
Methyl butanoate	623-42-7	748	755 [c]	MS, RI, STD	0.19[y]	0.15[y]	0.24[x]
Ethyl butanoate	105-54-4	825	831 [c]	MS, RI, STD	0.18[x]	0.12[y]	0.12[y]
Methyl hexanoate	106-70-7	951	951 [c]	MS, RI, STD	0.49[y]	0.43[y]	0.60[x]
Ethyl hexanoate	123-66-0	1037	1024 [a]	MS, RI, STD	0.05[y]	0.13[x]	0.03[y]
Methyl decanoate	110-42-9	1353	1358 [c]	MS, RI	0.44	0.5	0.57
Methyl dodecanoate	111-82-0	1550	1551 [d]	MS , RI, STD	0.34[y]	0.39[y]	0.52[x]
			Furans				
Furan	110-00-9	516		MS	0.04	0.07	0.04
2-Pentylfuran	3777-69-3	1015	1008 [a]	MS, RI, STD	0.08[z]	0.18[x]	0.14[y]
			Benzene/Phenols				
Benzene	71-43-2	685	682	MS, RI	0.29	0.26	0.29
Toluene	108-88-3	794	773 [a]	MS, RI	0.13	0.15	0.21
Ethylbenzene	100-41-4	892	883 [c]	MS, RI	0.05	0.06	0.06
p-Xylene	106-42-3	899	891 [c]	MS, RI, STD	0.14	0.13	0.15

Continued Table 6.4

o-Xylene	94-47-6	932	917 [c]	MS, RI, STD	0.08	0.05	0.07
Phenol	108-95-2	1110	1111 [c]	MS, RI, STD	0.07[x]	0.09[x]	0.05[y]
			Ketones				
Acetone	67-64-1	531	533 [b]	MS, RI, STD	0.44	0.6	0.56
2,3-Butanedione	431-03-8	629	631 [b]	MS, RI	2.23[x]	2.14[y]	1.80[y]
2-Butanone	78-93-3	637	639 [b]	MS, RI, STD	0.37	0.39	0.39
2-Pentanone	107-87-9	728	704 [a]	MS, RI, STD	0.61	0.73	0.8
2-Propanone,1-hydroxy-	116-09-6	732	709 [a]	MS,	0.36	0.4	0.35
3-Pentanone	99-22-0	735	740	MS, RI	0.21[y]	0.19[y]	0.29[x]
2-Hexanone	591-78-6	833	834	MS, RI	0.10[y]	0.16[x]	0.06[z]
2-Heptanone	110-43-0	935	931 [a]	MS, RI, STD	1.04	1.4	1.37
2-Octanone	111-13-7	1044	1039 [c]	MS, RI, STD	0.12[x]	0.14[x]	0.08[y]
2-Nonanone	821-55-6	1148	1141 [c]	MS, RI, STD	0.90[x]	0.99[x]	0.71[y]
2-Undecanone	112-12-9	1345	1349 [c]	MS, RI, STD	0.60[y]	0.59[y]	0.79[x]
2-Tridecanone	593-08-8	1544	1546 [a]	MS	0.91	0.95	0.93
2-Pentadecanone	2345-28-0	1749		MS	1.10[y]	1.21[y]	1.46[x]
			Lactones				
γ-Octalactone	104-50-7	1493		MS	0.15[x]	0	0
δ-Decalactone	705-86-2	1636	1638 [f]	MS, RI	0.23[y]	0.40[x]	0.34[x]
γ-6-(Z)-Dodecenolactone	18679-18-0	1799		MS	0.34[x]	0	0
γ-Dodecalactone	2305-05-7	1813		MS	1.19[x]	0	0
δ-Dodecalactone	713-95-1	1847		MS	1.07 [x]	1.04[y]	0.97[y]

Continued Table 6.4

Sulfurous Compounds							
Disulfide, dimethyl	624-92-0	779	754 [a]	MS, RI, STD	0.2	0.23	0.17
Terpenes							
D-Limonene	5989-27-5	1068	1045 [c]	MS, RI, STD	0.07[x]	0.05[y]	0.02[z]

Results are expressed as relative abundance normalized to internal standard.
CAS (Chemical Abstracts Service number). MS (identity confirmed by mass spectra to an in-house library)..
RI (linear retention index as determined). STD (identity confirmed by GC standard)
REF RI (relevant linear retention index as published reference, if available): [a] Clarke et al. 2022; [b] Vilar et al. 2021; [c] Olivares et al.2011; [d] GC standard; [e] Gianelli et al. 2011; [f] Garvey et al. 2021
Values in the same row not sharing the same superscript (x, y, z) specify significant difference ($P < 0.05$) in peak area value average.

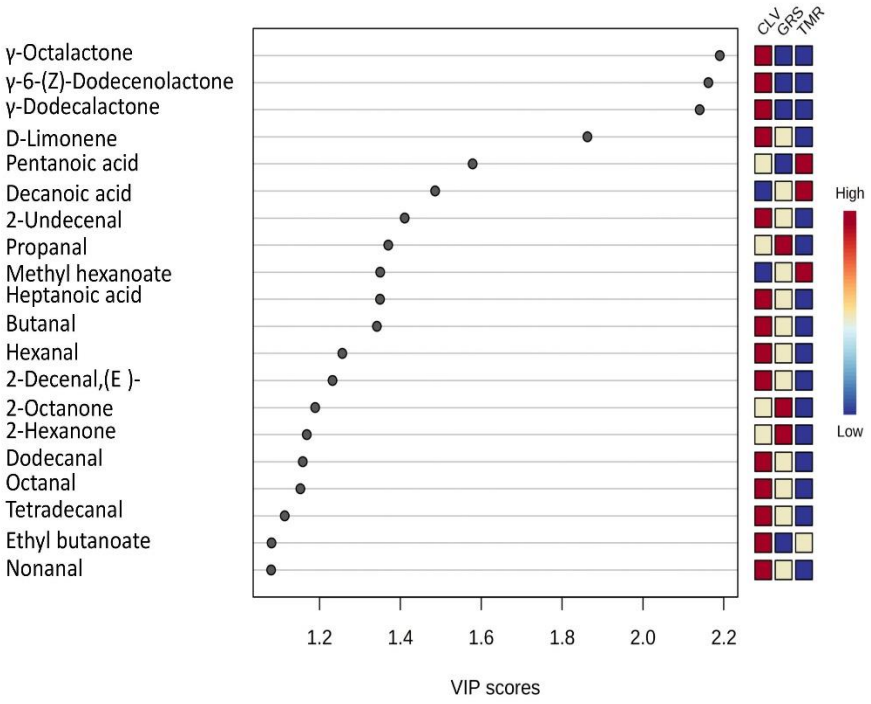


Figure 6.1 Variable Importance in Projection (VIP) plot shows the relevance of the most significant volatiles responsible for the differentiation between salted butter samples produced from different diets as determined by Partial Least Squares- Discriminate Analysis (PLS-DA). (CLV = salted butter derived from cows outdoors on perennial rye grass seeded with white clover), (GRS = salted butter derived from cows outdoors on perennial rye grass), (TMR salted butter derived from cows indoors on trial mixed rations).

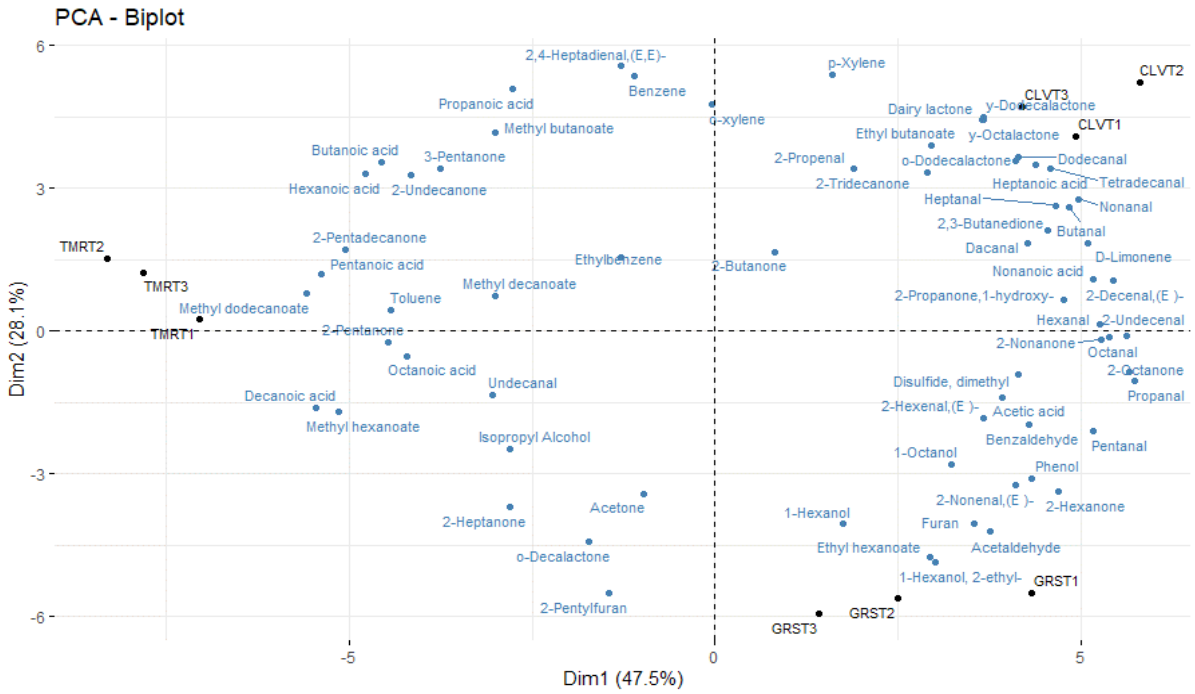


Figure 6.2 Principal component analysis plots highlighting the volatile relationship between the salted butters produced from CLV(salted butter derived from cows outdoors on perennial rye grass seeded with white clover), GRS (salted butter derived from cows outdoors on perennial rye grass), and TMR (salted butter derived from cows indoors on trial mixed rations). volatile compounds and relation between the different triplicate trials (T1, T2 and T3) of each type of salted butter based on volatile profiles.

6.3.3 Odor Characteristics of Aroma Active Compounds in Salted Butter as Determined by Gas Chromatography Olfactometry

In total 38 distinct odors were detected in these 3 salted butter samples, however we were unable to determine the source VOC for 14 of these distinct odors. This is not usual for GC-O, indicating the presence of an OAC but below the limits of detection by the MS (the aroma descriptions of these unidentified OAC were also included in Table 5). The largest OAC in terms of chemical class were aldehydes (8), followed by esters (5), then ketones (4), with one OAC from terpenes, furans, alcohols and sulphur compounds. Previous studies had identified 20 OAC in fresh cream butter by P&T (Peterson and Reineccius, 2003), or 32 in salted butter by either DHA or SAFE (Lozano et al., 2007) or 19 in heated butter by P&T (Peterson and Reineccius, 2003). Most odors were perceived in all three salted butter samples, with the exception of acetic acid, pentanal and unidentified 11, which were only perceived in GRS salted butter. The unidentified VOC 2 and 13 were only perceived in CLV and GRS salted butter, and unidentified VOC 3 was only perceived in CLV salted butter. Propyl butanoate was only perceived in CLV and TMR salted butter.In terms of the odor descriptions assigned to the OAC (minus the unidentified OAC) in relation to individual or co-eluted VOC by the trained panelists, in most cases these concur with previous studies (Peterson and Reineccus, 2003; Lozano et al., 2007; Mallia et al 2008a; Mallia et al., 2008b; Mallia et al., 2009) or by the goodscentcompany (http://www.thegoodscentscompany.com) (Supplementary Table 6.3). Those instances where identified OAC had different odor activities to those previously stated (nonanal, (E,Z) 2,6-nonadienal, (E)-2-nonenal, furfural, 2-furanmethanol , 2-octanone and 3-octen-2-one), likely indicates co-elution with another VOC that was above its odor threshold, but below limits of detection. Differences between VOC identified by

GC-MS (volatile profiling) and GC-O (olfactometry) in this study predominately relate to differences in the split ratio's for sample introduction to the GC (1:10 split for volatile profiling as opposed to splitless for the olfactory analysis) and the different column dimensions used for volatile profiling (DB-624 UI 60 m x 0.3 mm x 1.8 µm) and for olfactory analysis (DB-624 UI 20 m x 1.8 mm x 1.0 µm). The identity of 23 were confirmed through comparison of molecular ion matching, RI and analytical standards

Table 6.5 Odors including identified odor active compounds in salted butter.

Order of identification	Volatile compound	Retention Index		Odor Description	Odor Intensity(+, -)			Identification
		GC-O	REF RI		CLV	GRS	TMR	
1	2,3-Butanedione	571	574 [a]	Buttery, caramel	+	+	+	MS,RI, Odour,STD
2	Acetic acid	664	685 [a]	Vinegar	-	+	-	MS,RI, Odour, STD
3	Pentanal	709	733 [b]	Grassy	-	+	-	MS,RI, Odour, STD
4	Methyl butanoate	726	754 [c]	Fruity	+	+	+	MS,RI, Odour, STD
5	Disulfide, dimethyl	754	755 [a]	Sulphur, cabbage	+	+	+	MS,RI, Odour, STD
6	Unidentified-1	809	ND	Musty	+	+	+	Odour
7	Ethyl butanoate	816	831 [c]	Floral fruit	+	+	+	MS,RI, Odour, STD
8	Hexanal	828	830 [a]	Grassy	+	+	+	MS,RI, Odour, STD
9	Unidentified-2	845	ND	Cooked potato	+	+	-	Odour
10	Butanoic acid	880	878 [a]	Rancid	+	+	+	MS,RI, Odour, STD
11	Furfural	891	892 [a]	Nutty	+	+	+	MS,RI, Odour, STD
12	1-Hexanol	916	919 [b]	Grassy	+	+	+	MS,RI, Odour, STD
13	Propyl butanoate	922	921 [e]	Fruity	+	-	+	MS,RI, Odour, STD
14	Unidentified-3	930	ND	Musty	+	-	-	Odour
15	Heptanal	938	938 [a]	Grassy	+	+	+	MS,RI, Odour, STD
16	Unidentified-4	961	ND	Bread crust	+	+	+	Odour
17	Unidentified-5	977	ND	Musty, unpleasant	+	+	+	Odour
18	Unidentified-6	1008	ND	Roast	+	+	-	Odour
19	Benzaldehyde	1019	1020 [a]	Almond, mushroom-like	+	+	+	MS,RI, Odour, STD
20	Ethyl hexanoate	1024	1027 [a]	Fruity	+	+	+	MS,RI, Odour, STD

Continued Table 6.5

21	2-Octanone	1034	1039 [c]	Flower, hyacinth, metallic	+	+	+	MS,RI, Odour, STD
22	D-limonene	1043	1045 [c]	Citrus	+	+	+	MS,RI, Odour, STD
23	Octanal	1047	1048 [c]	Citrus, lemon	+	+	+	MS,RI, Odour, STD
24	Unidentified-7	1067	ND	Mushroom, grassy	+	+	+	Odour
25	Unidentified-8	1077	ND	Bread	+	+	+	Odour
26	3-Octen-2-one	1093		Musty	+	+	+	MS, Odour, STD
27	Unidentified-9	1119	ND	Painty, herb	+	+	+	Odour
28	Unidentified-10	1131	ND	Weak mushroom	+	+	+	Odour
29	2-Nonanone	1137	1140[d]	Burnt milk	+	+	-	MS,RI, Odour, STD
30	Unidentified-11	1140	ND	Fresh	-	+	-	Odour
31	Nonanal	1147	1148 [a]	Musty, damp	+	+	+	MS,RI, Odour, STD
32	Methyl octanoate	1152	1151 [b]	Sweet, fruity	+	+	+	MS,RI, Odour, STD
33	Unidentified-12	1164	ND	Cooked oil	+	+	+	Odour
34	Unidentified-13	1210	ND	Grassy, herbal	+	+	-	Odour
35	2-Nonenal,(E)-	1218	1218 [b]	Grassy	+	+	+	MS,RI, Odour, STD
36	Unidentified-14	1240	ND	Almond	+	+	+	Odour
37	Unidentified-15	1300	ND	Coconut fatty	+	+	+	Odour
38	Dodecanal	1451		Grass, green	+	+	+	MS, Odour, STD

Retention Index: GC-O (retention index as per gas chromatography olfactometry anlaysis).

REF RI found in the literature and reference: [a] Clarke et al. 2022; [b] Vilar et al. 2021; [c] Olivares et al.2011; [d] Garvey et al. 2021; [e] GC standard; ND, not detected.

Odor Intensity (OI):+, Odor description detected by five assessors during GC-O analysis. -, not detected. CLV (salted butter derived from cows outdoors on perennial rye grass seeded with white clover), GRS (salted butter derived from cows outdoors on perennial rye grass), TMR (salted butter derived from cows indoors on trial mixed rations.

Identification: MS (identity confirmed by mass spectra to an in-house library), RI (confirmed linear retention index), Odour (Odour intensity).

Supplementary Table 6.3 The odor descriptions for odor active compounds by panelists and references

		Reference					
OAC	GCO panelists	Goodscentscompnay	Peterson and Reineccius, 2003	Mallia et al., 2009	Lozano et al., 2007	Maillia et al., 2008a	Maillia et al., 2008b
Aldehydes							
Octanal	Citrus, lemon	Aledhydic, waxy, citrus, orange peel, green, herbal, fresh fatty		Almond, fat			
Nonanal [1]	Musty, damp	Mushroom-like, floral, green, waxy, fruity	Waxy, fatty, floral	Soapy, citrus	Mushroom	Waxy,fatty, floral	Soapy milk
2,6-Nonadienal (EZ) [1]	Grassy, herbal	Green, cucumber, melon, fatty, vegetable		Cucumber -like	Cucumber		
2-Nonenal (E) [1]	Grassy	Fatty, green, cucumber, aldehydic, citrus	Green, fatty	Hay	Hay	Green, fatty, tallowy	Green
Dodecanal	Grass, green	Soapy, waxy, aldehydic, citrus, green, floral					
Pentanal	Grassy	Apple		Fat, green			Fatty, perfume
Hexanal	Grassy	Green, slighty fruity, lemon, herbal	Fatty, grassy	Green	Green, grass	Green, fatty	Green, metalic
Furfural [1]	Nutty	Sweet, woody, almond, baked bread					
Heptanal	Grassy	Fresh, aldehydic, fatty, green, herbal, cognac		Soapy			Green, fatty
Esters							
Methyl octanoate	Sweet, fruity	Waxy, green, sweet orange, aldehydic, vegetable, herbal					
Ethyl ether	Solvent	Ethereal					
Methyl butanoate	Fruity	Fruity, apple, sweet, banana, pineapple		Fruity			Fruity
Ethyl butanoate	Floral, fruit	Fruity, pineabpple, cognac			Fruity, berry		

Continued Supplementary Table 6.3

Propyl butanoate	Fruity	Fruity, sweet, apricot, pineapple, rancid, sweaty					
Ethyl hexanoate	Fruity	Acidic, sweaty, cheesy, sharp, goaty		Orange-like			
Alcohols							
1-Hexanol	Grassy	Ethereal, fusel, oily, alcoholic, sweet, green					Soapy
2-Furanmethanol 1	Musty	Alocholic, chemical musty, sweet, caramellic, bready, coffee					
Ketones							
2-Octanone 1	Flower, hyacinth, metallic	Nice fruity, blue and parmesan cheese-like					
3-Octen-2-one 1	Musty	Earthy, spicy, herbal, sweet, mushroom, hay					
2,3-Butanedione	Buttery, caramel	Buttery	Buttery		Buttery	Buttery	Creamy
Acids							
Acetic acid	Vinegar	Sharp, pugent, sour vinegar			Vinegar		
Butanoic acid	Rancid	Rancid, cheesy, putrid, sweaty	Cheesy, rancid	Cheesy	Fecal, cheesy	Buttery, sweaty, cheesy, rancid	Cheesy, rancid
Sulphur							
Dimethyl disulfide	Sulphur, cabbage	Sulfurous, cabbage, vegetable, onion					Sulphur animal
Other							
Heptane	Solvent	Sweet, etheral					
Terpenes							
D-Limonene	Citrus	Citrus, orange, fresh, sweet				Citrus	Citrus green
Camphene	Bread crust	Woody, herbal, fir needle, terpenic, camphoreous					

Continued Supplementary Table 6.3

Unidentified					
1	Musty				
2	Cooked potato				
3	Musty, unpleasant				
4	Roast				
5	Mushroom, grassy				
6	Bread				
7	Painty, herb				
8	Weak mushroom				
9	Fresh				
10	Cooked oil				
11	Almond				
12	Coconut fatty				
Co-elution 2-Nonanone/furaneol 1	Burnt milk				
Benzaldehyde/1-octen-3-ol/y-butyrolactone	Almond, mushroom-like				
Descriptors for co-eluted compounds					
Furaneol 1	Burnt milk	Sweet, cotton candy, caramellic, strawberry, sugar	Sweet, caramel-like		
1-Octen-3-ol	Almond, mushroom-like	Mushroom, earthy, green, oily, fungal, raw chicken		Mushroom	Mushroom

Continued Supplementary Table 6.3

Benzaldehyde	Almond, mushroom-like	Sharp, sweet, bitter, almond, cherry	Almond-like, nutty	Roasted almond
2-Nonanone [1]	Burnt milk	Fresh, sweet, green, weedy, earthy, herbal		Milk
γ-Butyrolactone	Almond, mushroom-like	Creamy, oily, fatty		

[1]The odor active compounds identified by panelists with a different odor to the references.

6.4 Conclusion

This study has demonstrated that the optimized and validated DI-HiSorb GC–MS method was very suitable for the detection of VOC in salted butter and as a mechanism to concentrate VOC for olfactory analysis. Optimization of the design model was confirmed using the desirability factor (0.9109). The interaction effect of sample amount and extraction time significantly influenced the total area of all VOC in salted butter by DI-HiSorb extraction method. In total 66 VOC were identified in these salted butter samples and the abundance of 40 (~61 %) of these were significantly ($P < 0.05$) impacted by diet. These salted butter samples were easily discriminated from each other based on their volatile profiles. This study has highlighted that the impact of cow diet on the VOC profile of salted butter is even greater than previously envisaged and that CLV salted butter had the most complex volatile profile. The 3 most discriminating VOC present in these salted butter samples based on diet were lactones. This DI-HiSorb extraction method is very useful in targeting lactones which are often poorly extracted by other techniques such as HS-SPME. Thirty eight odors were perceived (although the source VOC of 14 were not identified) in all 3 salted butter samples, although the perception of 7 in terms of presence and absence were also impacted by diet.

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Chapter 7. Thesis overview and summary

7.1 Thesis Overview and Summary

7.1.1 Understanding the Relationship Between Volatile Compounds and Sensory Characteristics of Milk, Dairy powders, Yoghurt and Butter

Chapter 1 provides an up-to-date overview of the key aroma active VOC in milk, dairy powders, yoghurt, and butter, as well as their relationship to product quality from a flavour perspective, incorporating cross-cultural sensory analysis and new trends in sensory science and flavour chemistry applicable to these products. This review has highlighted advantages and/or shortcomings of different VOC extraction techniques in tandem with GS-MS, including complementary techniques such as GC-O. Combining sensory and analytical data using multivariate statistical analysis is essential in order to better understand flavour development in these products with a longer term aim of providing information to improve their quality and consistency. This review has also demonstrated the significance of product familiarity and how vital it is to understanding cultural factors that influence choice/acceptance, especially in countries with limited dairy product tradition.

7.1.2 Compare and Optimise Selected Volatile Extraction Techniques for Selected Dairy Products

A wide array of extraction techniques have been employed to isolate and concentrate VOCs from different dairy products, including, HS-SPME, TD, SBSE, SAFE and SDE. However, detection and quantification of VOC in dairy products is difficult due to the range of VOC of different chemical classes present and the inherent bias of each extraction techniques towards certain chemical classes. This PhD Thesis highlights the importance of optimising extraction techniques for individual dairy samples, and why multiple extraction techniques are often required in order to obtain as true a representation of the complete volatile profile.

HS-SPME linked to GC-MS is the most widely used volatile extraction technique for dairy products due to its versatility, high degree of automation, and due to the wide array of available absorbent/adsorbent phase materials, particularly for low molecular weight volatile compounds. In **Chapter 2** and **Chapter 3**, HS-SPME-was utilised with a multi-phase 50/30m DVB/ CAR/PDMS fibre to identify 26 and 24 VOCs in SMP and yoghurt, respectively. Even though this is a very popular extraction technique it has poor overall recovery of more polar compounds, especially free fatty acids, which are important volatile compounds in most dairy products. In **Chapter 4**, HS-SPME appeared to be quite efficient at recovering low boiling point VOC in WMP, such as terpenes and sulphur compounds, but was quite poor at recovering lactones, furans and as previously mentioned, acids. A major drawback of SPME is the limited capacity of the SPME fiber phase, which can result in competition between VOC resulting in limited recovery of these VOC with less affinity to the fiber phase. It's wide spread availability and ease of use however continues to ensure that it remains the most widely used extraction technique for dairy products.

TD is a well-established dynamic extraction process with a large variety of sorbent phases and is thus useful for a wide range of VOC with boiling points between 60 °C to 300 °C. In **Chapter 4**, TD was utilised using a combination of tenax and carbograph phases for volatile profiling of WMP. Tenax is ideally suited for the extraction of non-polar and slightly polar VOC (aldehydes, benzene/phenols, alcohols, and ketones), where carbograph is most suitable for the extraction of very low molecular weight VOC (six carbons or less). In this study TD did not extract lactones, acids or furans, but did recover aldehydes, ketones, alcohols and was particularly useful in recovering benzene/phenols.

High capacity sorptive extraction (HiSorb) is a relatively new VOC extraction technique available with a PDMS-coated probe. The benefits of HiSorb as a HS technique and especially as a DI technique where highlighted in **Chapter 4** in comparison to HS-SPME and TD for the analysis of WMP. The DI-HiSorb technique was especially useful in recovering lactones, which are a class of VOC poorly recovered by many other extraction techniques, but yet important aromatic compounds in many dairy products. This aspect was further highlighted in **Chapter 5** and **Chapter 6** for recovering volatiles in WMP and butter.

The importance of GC column polarity in VOC extraction was also highlighted in **Chapter 4**, where a non-polar column considerably out preformed a polar column in terms of the number of VOC identified from the same volatile extracts. This is important for dairy products where the nature of specific chemical classes will be similar even between different dairy products. The impact of salting out was also evaluated in this same study, and its overall impact was negligible in comparison to GC column polarity, however the effectiveness of salting out very much depends upon the composition of the sample and the conditions of the extraction.

7.1.3 Key Volatile Compounds in Yoghurt, Butter and Dairy powders

Many similar VOC compounds are present in different dairy products, as they come directly from the milk or are created from substrates in the milk through bacterial metabolism and / or thermal treatments. This study has identified very abundant VOC in yoghurt, SMP, WMP and butter, which are also likely to be the key VOC in these products. 2,3-Butanedione (diacetyl), acetone, acetoin and 2,3-pentanedione was very prominent in yoghurt (**Chapter 3**), as they can be generated from glycolysis or citrate metabolism of several LAB (*S. thermophilus,* and *Lb. bulgaricus* strains). Likewise

some other volatile compounds, such as methyl ketones (2-heptanone, 2-butanone, 2-pentaneone, 2-hexanone, 2-octanone, 2-undecanone) and aldehydes (2,4-decadienal, pentanal, hexanal, heptanal, octanal, nonanal, undecanal, 2-nonenal-(E), benzaldehyde) which are products of lipid oxidation and were also present in yoghurt in this study . Some sulphur (dimethyl disulphide), terpenes (α-pinene), benzenses (toluene, benzene, p-xylene) were also very abundant. The aroma profile produced during fermentation can be significantly influenced by even slight variations in the composition of the starter cultures. WMP (**Chapter 4** and **Chapter 5**) in this study was characterised by a high abundance of lactones (mainly δ-decalactone and δ-dodecalactone), methyl ketones (2-pentanone, 2-heptanone, 2-butanone, 2-nonanone), aldehydes (heptanal, nonanal, decanal) and 2-ethyl-1-hexanol, and slightly lower levels of terpenes (α-pinene, 3-carene), aldehydes (3-methyl-butanal, hexanal, octanal, benzaldehyde), 1-pentanol, methyl butanoate, toluene, p-xylene amongst others. SMP (**Chapter 2**) was characterised by a high abundance of dimethyl sulfone, acetone, 2-ethyl-1-hexanol and butanoic acid. Acetoin was found to be the most discriminatory compound based on abundance levels in SMP produced from milk derieved from different cow different diets and might be product of hetero-lactic fermentation combined with citrate formation by lactic acid bacteria in cow rumen. However, a range of other alcohols (ethanol, 1-butanol, benzyl alcohol), aldehydes (hexanal, heptanal, nonanal, benzaldehyde), ketones (2-butanone, 1-hydroxy-2-propanone), terpenes (α-pinene, 3-carene), and ε-caprolactone, acetic acid and phenol were also very prominent. Butter **(Chapter 6)** was characterised by high abundance of acids (pentanoic, decanoic and heptanoic acids), ketones (2-octanone and 2-hexanone), lactones (γ-octalactone, (Z)-dairy lactone and γ-dodecalactone), aldehydes (2-undecenal, propanal, butanal, hexanal, dodecanal, octanal, (E)-2-decenal, tetradecanal

and nonanal), esters (methyl hexanoate, ethyl butanoate) and the terpene (D-limonene).

Most of the aldehydes present in these yoghurt, SMP, WMP and salted butter samples are likely derived from lipid oxidation or Strecker reactions, with ketones derived either from carbohydrate metabolism or lipid oxidation. Most of the alcohols are also secondary products of lipid oxidation, or in minority of cases from carbohydrate metabolism or possibly Strecker reactions. Most short chain acids are a direct result of lipolysis or from carbohydrate metabolism, with esters created from short chain acids and alcohols. The lactones are derived from fatty acids, the abundance of which are often enhanced like some ketones by any thermal processing. The benzene or phenolic compounds are likely derived from the metabolism of β-carotene or flavonoids (or also Strecker reactions). Terpenes have either been directly transferred from the diet or metabolised by bacteria from larger sesquiterpenes.

7.1.4 The Association Between Volatile Compounds, Sensory Properties Based on Bovine Diet

The VOC profiles of SMP from GRS, CLV and TMR diets were quite distinct (**Chapter 2**), although the volatile profiles of SMP from GRS and CLV were more closely related. The VOC most impacted by diet in SMP were predominantly metabolic carbohydrate products such as acetoin and acetic acid, which were highest in TMR SMP and lowest in CLV SMP. Both GRS SMP and CLV SMP had greater levels of dimethyl sulfone, heptanal, pentanal and 2-undecanone arising from Strecker reactions and lipid oxidation. SMP from CLV was found to have a higher scores for "cardboard/wet paper" and "cowy/barny" than the other SMP products and it was noted that the lipid oxidation aldehydes pentanal and heptanal often associated with cardboard flavour were also significantly higher in this SMP. It was suggested that the

higher score for “cowy/barny” odour may be due to phenolic compounds often associated with pasture-fed dairy products, although not detected in this study. Other panellists found that “oxidised flavour” was greatest in SMP from TMR and both the lipid oxidation aldehydes nonanal and decanal were highest in this SMP.

The volatile profiles of salted butter from GRS, CLV and TMR diets were also quite distinct, highlighting again the impact of diet **(Chapter 6)**. Most of the differences in VOC were related to fatty acid content as they were either mainly products of lipid oxidation (aldehydes or ketones), short chain fatty acids and esters or lactones. Propyl butanoate (fruity aroma) was only detected in salted butter samples from CLV and TMR. On the other hand, the compounds unidentified-2 (cooked potato), unidentified-6 (roast), 2-nonanoe (burnt milk), and unidentified-13 (grassy, herbal) were only detected in CLV and GRS salted butter samples. There were no odors perceived in TMR salted butter that were not also detected in GRS or CLV salted butter.

Again very distinct differences in the volatile profiles of WMP were apparent based on diet, with WMP from GRS and CLV more similar (**Chapter 5**). Seventeen of the twenty six VOCs that were significantly different based on diet in these WMP samples were derived from fatty acids either by lipid oxidation, thermal degradation or β-oxidation and lactonization (butanal, pentanal, hexanal, hexanal, 1-pentanol, 1-hexanol, 2-nonanone, 2,3-octanedione, δ-hexalactone, δ-octanolactone, δ-nonalactone, δ-decalactone, δ-undecalactone, δ-dodecalactone, δ-tridecalactone, γ-dodecalactone) which is similar to the trend found with the salted butter samples. The potentially most significant differences in VOC due to diet were the lactones, were both δ-decalactone and δ-dodecalactone were significantly more abundant in WMP

derived from CLV and GRS diets, with the abundance of γ-dodecalactone significantly higher in WMP from TMR diets.

These studies highlight that cow diet significantly affects the volatile profile of SMP, WMP and salted butter, with pasture-diets (GRS and CLV) more similar than TMR diets. Similar trends were found in WMP and salted butter in that many of the differences in VOC were either directly or indirectly linked to fatty acids, which are known to be significantly impacted by diet. It also appears that these differences ar exacerbated by thermal treatments during product processing. The trends in terms of the impact of fatty acids was not as clearly evident for SMP, however this is most likely to due the extraction technique used, as HS-SPME containing the triple fibre has poor recovery of more polar volatile compounds, such as fatty acids or lactones. It may also be due to the low fat content and processing mechanisms used to produce SMP.

7.1.5 The Cross-cultural Sensory Differences of Selected Dairy Products

There seems to be a shortage of cross-cultural sensory research, with little or none undertaken on Irish dairy products. The results from this study demonstrated the importance of product familiarity in relation to consumer acceptance and liking in SMP and yoghurt. Export-oriented Irish dairy producers can benefit from scientific understanding generated in **Chapter 2** and **Chapter 3** regarding factors influencing customer preference and how these compare to a reference Irish consumer group, in order to maximise market penetration and reduce potential market expansion hurdles.

In **Chapter 2**, cross cultural sensory and volatile analysis was undertaken on SMP produced from milk derived from three distinct feeding systems; pasture based feeding (CLV, GRS) and concentrate based feeding (TMR) systems. Cross cultural

sensory analysis was undertaken in Ireland, China, and the USA to determine if sensory attributes could be correlated with specific VOC. Consumers in the USA, China, and Ireland did not vary significantly in their preference for SMPs derived from CLV or GRS diet. However, USA customers significantly preferred SMP produced from concentrate (TMR) feeding system for "aroma liking", "overall liking", "flavour liking", "freshness intensity", "appearance liking", "creaminess liking", and "aftertaste liking". This also demonstrates that preference is based on familiarity, since most dairy products in the USA are produced from cows fed TMR indoors rather than from a pasture based feeding system. With the exception of "appearance-liking", "cooked flavour intensity" and "aftertaste intensity", Irish customers preferred pasture-fed (CLV and GRS) SMP over TMR-fed SMP and scored it higher on most attributes. Chinese consumers were less discerning in relation to diet, but both Chinese consumers and trained assessors scored many attributes quite differently than their USA or Irish counterparts, likely reflecting a lack of familiarity with dairy products in general. Significant cultural differences exist in the "aftertaste like" characteristic of Chinese consumers, which may be due to the cultural ambiguity of this characteristic. Before conducting any cross-cultural sensory evaluation, this study also emphasises the necessity of validate scales and any questions to avoid misunderstandings in sensory attributes carried on by conceptual differences across cultures. This will improve the validity and reliability of cross-cultural sensory and consumer results.

In **Chapter 3**, Irish yoghurt products were produced in triplicate trials from 3 different starter culture mixtures. Cross-cultural hedonic analysis and ODP sensory analysis was undertaken in Ireland and Germany in combination volatile analysis by HS-SPME GCMS. Both Germany and Irish consumers were unable to distinguish between the three yoghurt products in terms of appearance, aroma, flavour, and

texture. However, Irish consumers scored “liking of appearance”, and “overall acceptability” significantly higher than German consumers for all samples. Irish consumers also scored one sample higher for “liking of texture” and two others higher for “liking of flavour” than the German consumers. In OPD assessment German assessors did not find any difference between the samples, but Irish assessors found significant differences between all 3 yoghurt types. It is possible that the differences detected between both cultures may also be linked to familiarity with Irish yoghurt products. German consumers and assessors would be less familiar with yoghurt produced from pasture based milk production systems and thus may be less able to discern differences than Irish consumers and assessors.

7.2 Future Recommendations

Understanding the volatile aroma profile of Irish dairy products provided insight into the flavour and aroma formation reactions that impact sensory perception. Combining multiple sensory approaches, volatile profiling, and olfactometry is the most effective method to understand key VOC and factors influencing their formation.

Considering the results of this thesis, the following considerations for future research are recommended:

- More research is required in relation to volatile compounds associated with pasture-feeding that may impact on sensory perception. This is especially the case for those VOC potentially responsible for “barnyard flavour or aroma” which are much more prominent in many dairy products from pasture-based feeding systems but also more easily perceived by USA consumers, assessors and trained panellists. It has been proposed that p-cresol is likely responsible but it may also be other phenolic compounds. The fact that p-cresol is also linked to the presence of white

clover in the diet and that white clover is proposed as a solution to bind more nitrogen and reduce fertiliser usage on pasture in Ireland, it would seem logial that more research is undertaken in this areaA more in-depth study is required investigating flavonoid content in the rumen and correlating this to white clover content in the diet. Even though most of these phenolic compounds are excreted, some are transferred to the milk and as they are extremely odour active, small increases are likely to have a significant impact on sensory perception, which is exacerbated by cultural familiarity to pasture based dairy products. Utilising advances in VOC extraction in conjunction with comprehensive gas chromatography (GCxGC) high resolution mass spectrophotometry offers the best opportunity to identity and quantify these trace level phenolic compounds.

- Some of the cross cultural research undertaken in this thesis has also highlighted potential issues in relation to potential differences in definitions of specific sensory attributes/lexicons on a cultural basis. We are suggesting that it is important to investigate the potential of new non-verbal sensory methodologies, behavioural assessments, and sensometrics to increase the validity and reliability of cross-cultural sensory research, and avoid issues around ambiguity. This is particularly important in relation to Irish dairy products produced from a pasture based dairy system that is relatively unique globally.
- As mentioned advances in VOC extraction and enhanced separation capability of GCxGC with improved detection sensitivity can greatly aid in determining key VOC influencing sensory perception. Recently advances have also been made in understanding the syngerstic impact of two or more VOC in relation to specific aromas not associated with a single VOC. This is achieved through sensory omission studies using olfactometry type techniques and offers great potential to

unravel the complex aromatic properties of dairy products. Such research can greatly aid our understanding of the uniqueness of Irish dairy products but also help us target key markets or target those markets more likely to prefer Irish dairy products.

Appendix

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A cross-cultural sensory analysis of skim powdered milk produced from pasture and non-pasture diets

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ABSTRACT

Understanding potential cross-cultural sensory differences in the perception of Irish dairy products is important for key markets such as the USA and China. As most Irish dairy products are produced from pasture derived milk, this study investigated the impact of pasture and non-pasture diets on the cross cultural sensory perception of skim milk powder (SMP) in Ireland, USA and China. SMP was produced from cows fed outdoors on ryegrass (GRS), ryegrass/white clover (CLV), and indoors on trial mixed rations (TMR). SMP samples were evaluated by Irish (n = 78), USA (n = 100) and Chinese (n = 106) consumers using an identical hedonic sensory acceptance test in Ireland, USA and China. Optimized Descriptive Profiling (ODP) was performed using trained assessors familiar with dairy products in Ireland (n = 25) and China (n = 22), and traditional descriptive analysis was undertaken by a trained panel (n = 7) in the USA. Volatile analysis was undertaken on each SMP sample. Hedonic assessment found that USA consumers preferred SMP derived from TMR, and Irish consumers preferred SMP from either GRS or CLV. Chinese consumers perceived SMP samples differently to the USA and Irish consumers, but preference was not influenced by diet. Both Irish and Chinese trained assessors found it more difficult to discern differences between GRS or CLV SMP, but could differentiate TMR SMP. Irish assessors preferred GRS and CLV SMP. Chinese and Irish assessors had different preferences for many attributes. Trained USA panelists found significant differences, exclusively associating pasture based diets with "cowy/barny" and "cardboard/wet paper" attributes and more intense "grassy/hay" attributes than in TMR SMP. The abundance of ten volatile compounds differed significantly based on diet with acetoin derived from carbohydrate metabolism at much greater abundance in TMR SMP. This study found that sensory perception and volatile profiles of SMP were influenced by diet and differences in sensory perception existed between the three cultural groups. Irish and USA sensory responses aligned with familiarity of dairy products derived from pasture and non-pasture diets, respectively, and Chinese sensory responses differed to Irish and USA responses likely reflecting their lack of familiarity with dairy products.

1. Introduction

There is an increased demand for pasture-fed dairy products resulting from consumer perceptions of a healthier, "added value" and "more natural" products than those derived from indoor feeding systems (Kilcawley, Faulkner, Clarke, O'Sullivan, & Kerry, 2018). From a nutritional point of view, bovine milk derived from pasture feeding provides better protein, omega-3, and vitamins than from conventional indoor ration

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feeding systems (Elgersma, 2015; Egan, Galvin, & Hennessy, 2018). Ireland has an ideal temperate climate and soil type for grass growth and thus pasture feeding systems (Dillon, Roche, Shalloo, & Horan, 2005). As the Irish dairy sector is export driven, it is incumbent to have a greater understanding of the factors influencing sensory perception in key global markets. Different food environments and dietary experiences across cultures are known to influence both sensory perception and consumer behaviour (Prescott & Bell, 1995). Therefore, the understanding of food familiarity by consumers and cultural differences associated with food perception is vital in sensory research to understand differences in consumer behaviour (Soares et al., 2017).

The familiarity of food products plays a vital role in the acceptability and preferences of consumers because it delineates cultural differences in food, as consumers tend to favour familiar food (Torrico, Fuentes, Viejo, Ashman, & Dunshea, 2019). Familiarity is acquired through previous experience and repeat exposure which decreases the uncertainty with regard to safety and identity and thus has a positive impact on liking (Methven, Langreney, & Prescott, 2012). A recent study by Kim, Petard, and Hong (2018) demonstrated that familiarity affected consumers' perception of nuttiness in soymilk amongst Chinese, Korean and Western consumers. In another study of Mexican and Brazilian nopal (Opuntia cacti) consumers, differences in acceptance and perception were found to be mainly due to differences in familiarity rather than natural personal variations (de Albuquerque et al., 2018). Moreover, Kim et al. (2018) reported that French and Korean trained panels showed different preference patterns according to familiarity especially when evaluating unfamiliar green tea products.

Previous studies have highlighted that the feeding system of dairy cows can affect the composition of milk with pasture derived milk having significantly higher proportions of unsaturated fatty acids (UFA) and conjugated linoleic acid (CLA) compared with those derived from total mixed ration (TMR) diets (Coakley et al., 2007). Feeding systems have also been shown to alter volatile compounds in bovine milk as these can transfer directly, and indirectly through rumen metabolism from forage into milk (Kilcawley et al., 2018; Martin, Verdier-Metz, Buchin, Hurtaud, & Coulon, 2005). Volatiles derived from the metabolism of dietary components can also have a sensory impact; "barnyard" aroma/flavor appears to be linked to *p*-cresol content in milk (Faulkner et al., 2018; Martin et al., 2005). Croissant, Washburn, and Drake (2007) documented sensory flavor differences by both trained panelists and consumers between milk from grass fed cows and milk from cows fed a TMR diet. In the sensory evaluation of milk and cheese, differences in visual (mainly color) attributes appear easier to discern than flavor differences by both trained and untrained panels (Kilcawley et al., 2018). The feeding system can also affect the natural color of products, and TMR diets produce dairy products that are whiter in color than those of pasture feeding systems, which have a characteristic yellow color because of the higher carotenoid (β-carotene) content (Hurtaud et al., 2002, O'Callaghan et al., 2016a).

Dairy powders are major commodity export products and this study assessed consumer perceptions of bovine skim milk powder (SMP) from three feeding systems; TMR diet indoors, perennial ryegrass outdoors and perennial ryegrass/white clover outdoors in Ireland, China and USA. Volatile analysis was also undertaken to determine if sensory attributes could be chemometrically associated with specific volatiles.

Table 1
Sensory terms for optimised descriptive analysis of skim milk powder.

Descriptor	Explanation	Scale
Intensity		
Appearance-Colour	Appearance-Ivory to orange colour	0 = Pale, 10 = Yellow
Sweet Aroma	The smell associated with dairy sweet milky products	0 = none, 10 = extreme
Creamy Aroma	The smell associated with creamy/milky products	0 = none, 10 = extreme
Cooked Aroma	The smell associated with cooked milk products	0 = none, 10 = extreme
Oxidised Aroma	The smell associated with rancid or oxidised products	0 = none, 10 = extreme
Painty Aroma	The smell associated with rancid paint type notes	0 = none, 10 = extreme
Chalky Texture	Chalk like texture in the mouth	0 = none, 10 = extreme
Powdery Texture	Powdery texture in the mouth	0 = none, 10 = extreme
Viscosity	Thick texture in the mouth	0 = none, 10 = extreme
Sweet Taste	Fundamental taste sensation of which sucrose is typical	0 = none, 10 = extreme
Sour Taste	Fundamental taste sensation of which lactate is typical	0 = none, 10 = extreme
Salty Taste	Fundamental taste sensation of which NaCl is typical	0 = none, 10 = extreme
Creamy Flavor	The flavour associated with creamy/milky products	0 = none, 10 = extreme
Dairy Sweet Flavor	The flavours associated with sweetened cultured dairy products such as fruit yoghurt	0 = none, 10 = extreme
Carmelized Flavor	The flavour associated with caramel	0 = none, 10 = extreme
Oxidised Flavor	The flavour associated with rancid or oxidised products	0 = none, 10 = extreme
Rancid Butter	The flavour associated with rancid or oxidised butter	0 = none, 10 = extreme
Painty Flavor	The flavour associated with rancid paint type notes	0 = none, 10 = extreme
Grassy Flavor	The flavours associated with cut grass or hay	0 = none, 10 = extreme
Cooked Flavor	The flavour associated with cooked milk products	0 = none, 10 = extreme
Off-flavor	Off-flavour (Rancid)	0 = none, 10 = extreme
Astringent After-taste	Fundamental taste sensation of which aluminium sulphate is typical	0 = none, 10 = extreme

2. Materials and methods

2.1. Skim milk powder manufacture

In this study SMP was evaluated as it remains a considerable dairy export product for Ireland and has a longer shelf life than whole milk power. Raw milk from 54 Friesian cows was split into three groups (n = 18) at the Teagasc Moorepark dairy farm, Fermoy, Co. Cork, Ireland. Each group of 18 cows were given separate diets, (A) outdoors on perennial rye-grass only (GRS), (B) outdoors on perennial ryegrass/white clover (CLV) and (C) indoors on TMR as described by O'Callaghan et al. (2016b).

Raw whole milk (approximately 1000 kg) was collected from the cows on each dietary treatment. Milk was pre-heated to 50 °C in an APV plate heat exchanger (SPX Flow Technology, Crawley, West Sussex, UK), separated by a centrifugal disk separator, and pasteurized at 72 °C for 15 s. The pasteurized milk was subsequently preheated to 78 °C and evaporated in Niro three-effect falling film evaporator (GEA Niro A/S, Soeborg, Denmark) at sequential effect temperatures of 73 °C, 64 °C and 55 °C. Concentrate feed introduced to Niro Tall-Form Anhydro three-stage spray dryer (GEA Niro A/S, Soeborg, Denmark) (air inlet temperature = 180 °C, air outlet temperature = 85 °C) at approximately 43% total solids (TS) with a centrifugal atomizer (GEA Niro A/S, Soeborg, Denmark) at Moorepark Technology Ltd, Fermoy, Co. Cork, Ireland. Primary and secondary fluidized beds were maintained at 74 °C and 24 °C, respectively. Fines were returned to the cyclone to the top of the spray dryer. Yielding low-heat non-agglomerated SMP of approximately 97% total solids, and a fat content <0.1%.

2.2. Consumer acceptance testing

2.2.1. Consumers

Consumers residing in Fuzhou (China, n = 106), Raleigh (USA, n =

Table 2
Sensory language for descriptive analysis of rehydrated skim milk powders.

Term	Definition	Reference	Example/preparation
Aroma Intensity	The total orthonasal aroma impact		Evaluated as the lid is removed from the cupped sample
Sweet Aromatic	Sweet aromatics associated with dairy products	Vanillin in milk	Vanilla cake mix or 20 ppm vanillin in milk
Cooked/ Milky	Aromatics associated with cooked milk	Cooked skim milk	Heating skim milk to 85 °C for 30 min
Cardboard/ Wet paper	Aromatics associated with wet cardboard or paper	Cardboard paper	Brown paper bag cut into strips and soaked in water
Grassy/Hay	Aromatics associated with dried grasses	Alfalfa or grass hay	
Sweet Taste	Fundamental taste sensation elicited by sugars	Sucrose	Sucrose(5% in water)
Salty Taste	Fundamental taste sensation elicited by salts	Sodium chloride	Sodium chloride (0.5% in water)
Astringent	Fundamental taste sensation elicited by aluminium sulphate	Aluminum sulphate	Aluminum ammonium sulfate (0.09% in water)
Cowy/Barny	Aromatics associated with cow feces and urin	p-cresol	20 ppm p-cresol in skim milk

100), and Cork (Ireland, n = 78) having similar characteristics in terms of gender, occupation (student) and age (18–30 years), participated in the consumer acceptance study (hedonic attribute testing). Consumers were regular self-reported consumers of milk, had experiences in drinking powdered milk products, and were non-rejecters of milk.

2.2.2. Evaluation procedure

Milk powder samples were rehydrated at 10% solids (w/v) and stored at 4 °C overnight to allow powders hydrate. Preparations were conducted with the overhead lights off to prevent light-induced off-flavor formation. Consumers used the sensory hedonic attributes provided to them for the three different SMP samples (TMR, GRS, and CLV) presented three times. For consumer testing, samples were dispensed into 30 ml Styrofoam tumblers with three digit codes and taken from the refrigerator (4 °C) and served after 15 min at ambient temperature (20 °C). Each sample was served in a randomised balanced order with a 1 min rest between each sample. Consumers were asked to assess using a 9-point hedonic scale the liking of the following attributes: "appearance", "aroma", "overall", "flavor", "mouthfeel/thickness", "creaminess" and "aftertaste" (hedonic). The consumers also rated the intensity of "freshness", "cooked flavor", aftertaste" and "quality" using a 5-point category scale anchored on the left with extremely low and on the right with extremely fresh (Stone, 2012). Sensory data was collected on paper ballots in Cork, Ireland and Fuzhou, China and by computerized data entry at Raleigh, USA.

2.3. Descriptive analysis

2.3.1. Optimized descriptive profiling

Optimized Descriptive Profiling (ODP) (Silva et al., 2012, 2013; Silva, Minim, Silva, Peternelli, & Minim, 2014) was only undertaken with panels in China and Ireland. ODP was chosen as a fully trained descriptive panel was not available in China. Assessors were trained (O'Sullivan, 2016) in Fujian Agricultural and Forest University, China (n = 22) and at University College Cork, Ireland (n = 25). These assessors were presented with all samples simultaneously but with randomised order to prevent first order and carry-over effects (MacFie, Bratchell, Greenhoff, & Vallis, 1989). Assessors used the consensus list of sensory descriptors which were measured on a 10 cm line scale with the term "none" used as the anchor point for the 0 cm end of the scale and "extreme" for the 10 cm end of the scale (Table 1). For this study training and the use of a consensus sensory lexicon were used as described by Richter, de Almeida, Prodencio, and Benassi (2010) and Faulkner et al. (2018) for a Ranking Descriptive Analysis technique. Sensory terms, which were the main sensory dimensions, were pre-selected from the sample set using an expert sensory panel (n = 10). Assessors evaluated the intensity of each attribute for each sample on the scales. Attributes were presented along with the table describing the sensory terms (Table 1). All samples were prepared in the same manner as the consumer analysis study and presented in triplicate.

2.3.2. Descriptive analysis

Descriptive sensory analysis (DA) was undertaken in the USA only. Rehydrated milk powders were evaluated in duplicate by seven trained panelists (Sensory Service Centre, North Carolina State University, USA), each with more than 120 h of experience in the descriptive analysis of dried dairy ingredients. Panelists evaluated the rehydrated milk powders using an established sensory lexicon (Table 2) and an intensity scale consistent with the Spectrum descriptive analysis method (Drake, Karagul-Yuceer, Cadwallader, Civille, & Tong, 2003; Lloyd, Drake, & Gerard, 2009). All samples were prepared as per the consumer analysis study and presented in duplicate. In separate sessions, panelists evaluated coded samples in duplicate according to appropriate sensory practices. For analysis, each panelist evaluated the aroma of the sample and then tasted each sample. Samples were expectorated, and deionized water was used for palate cleansing.

2.4. Volatile analysis

The SMP samples were analysed by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME GCMS) in triplicate. Four g of SMP sample (rehydrated at 10% solids (w/v)) was added to 20 ml amber screw capped headspace vials (Apex Scientific, Maynooth, Ireland) and equilibrated to 40 °C for 10 min with pulsed agitation of 5 sec at 500 rpm using a Shimadzu AOC 5000 Autosampler (Mason Technology Ltd, Dublin, Ireland). A single SPME fibre 50/30 μm Carboxen™/divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS), (Agilent Technologies Ltd, Cork, Ireland) was exposed to the headspace above the samples in the vial for 20 min at a depth of 1 cm at 40 °C. The fibre was retracted and injected into the gas chromatograph inlet and desorbed for 2 min at 250 °C. Injections were made on a Shimadzu 2010 Plus GC (Mason Technology Ltd, Ireland) with an DB-624 UI (60 m × 0.32 mm × 1.80 μm) (Agilent Technology Ltd, Ireland) column using a split/splitless injector in splitless mode with a merlin micro seal (Agilent Technology Ltd, Ireland). The temperature of the column oven was set at 40 °C, held for 5 min, increased at 5 °C min^{-1} to 230 °C then increased at 15 °C min^{-1} to 260 °C, yielding at total run time of 65 min. The carrier gas was helium held at a constant flow of 1.2 ml min^{-1}. The detector was a Shimadzu TQ8030 mass spectrometer detector (Mason Technology Ltd, Ireland), ran in single quad mode. The ion source temperature was 220 °C and the interface temperature was set at 260 °C. The mass spectrometer mode was electronic ionization (70v) with the mass range scanned between 35 and 250 amu. Compounds were identified using mass spectra comparisons to the NIST 2014 mass spectral library, a commercial flavor and fragrance library (FFNSC Shimadzu, Mason Technology Ltd, Ireland) and an in-house library created in Shimadzu GCMS Solutions software (Mason Technology Ltd, Ireland) with target and qualifier ions and linear retention indices for each compound. Linear retention indices were performed as per the method of Van den Dool and Kratz (1963). Spectral deconvolution was also performed to confirm the identification of compounds using AMDIS software (v 2.3, Gaithersburg, MD, USA). Ten ul of a standard solution (1-butanol, dimethyl disulphide, butyl acetate, cyclohexane, benzaldehyde) at 10 ppm, and 2-phenylethanol at 50 ppm in methanol: water (1:99) were run before and after every series of samples to ensure that both the SPME extraction and MS detection were performing within specification. An auto-tune of the GCMS was carried out prior to the analysis to ensure

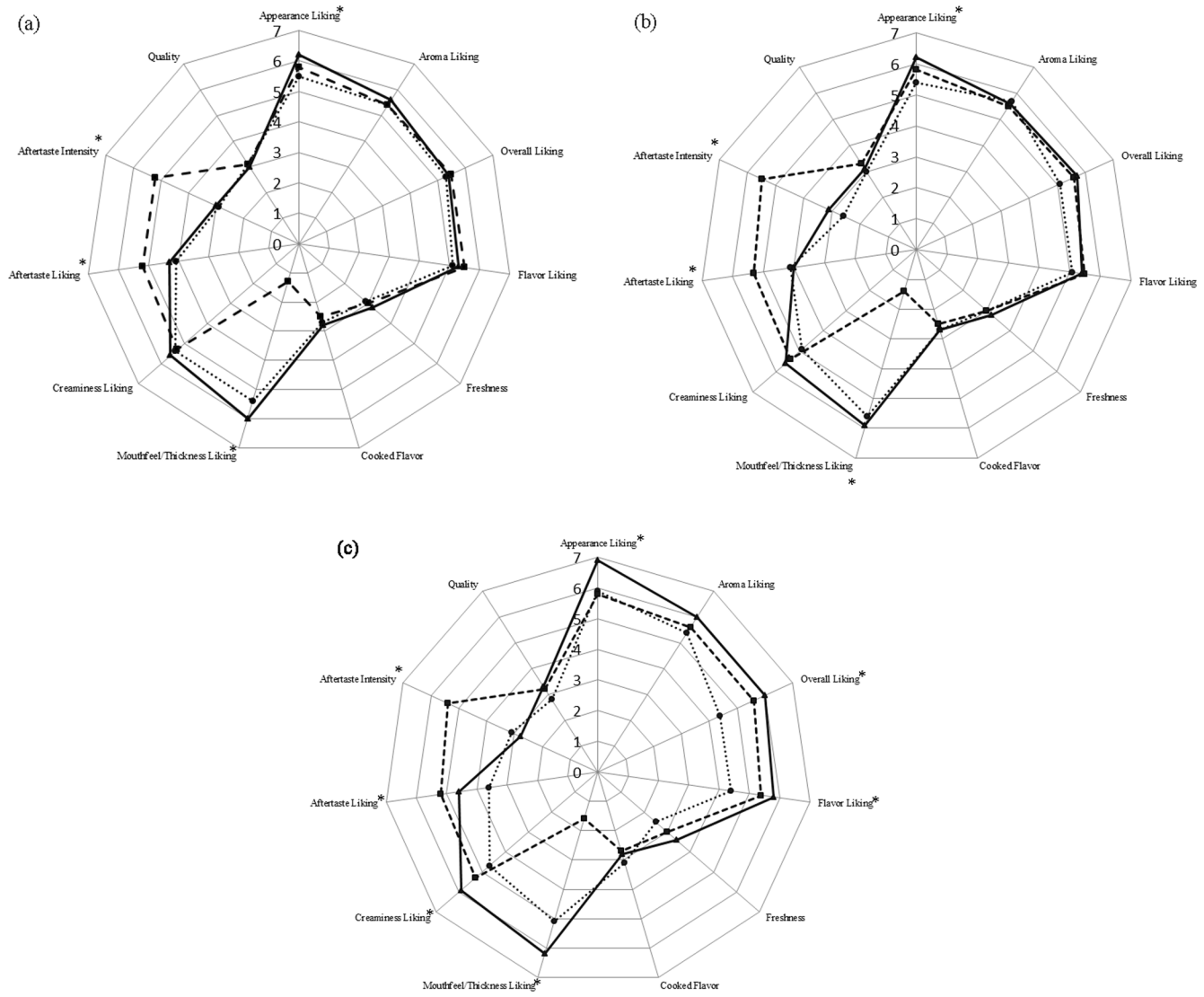

Fig. 1. Consumer acceptance study performed by Irish (●), Chinese (■) and USA (▲) consumers' on reconstituted skim milk power derived from three distinct diets; (a) clover (CLV), (b), grass (GRS), and (c) total mixed ration (TMR). Significantly different attributes are marked with an asterisk ($p < 0.05$).

optimal performance. Results were expressed as abundance values only which represent peak areas, due to the difficulty of quantification using HS-SPME where multiple chemical classes are present in the sample.

2.5. Statistical analysis

The statistical analysis of the volatile compounds was undertaken using Kruskal-Wallis- Partial Least Squares Regression (KW-PLSR). PSLR combines features of Principal Component Analysis (PCA) and multiple regressions and is performed separately on a set of dependent variables from a large set of independent variables. Based on the PSLR Beta coefficients results, the compound which has a significantly higher ($p < 0.05$) absolute value of the beta coefficient (Arjo, 2009) was used in the Kruskal-Wallis H test (one-way ANOVA on ranks) to evaluate the significant differences among the SMP samples for each volatile compound. To classify SMP samples in a supervised multivariate model, partial least squares discriminant analysis (PLS-DA) was performed. The Variable Importance Plot (VIP) scores summarised the individual X variables and have an influence on the PLS model and rank in terms of importance on the Y axis (with variables of the highest importance at the top) (Strobl, Boulesteix, Zeileis, & Hothorn, 2007). VIP scores give a measure useful to show the volatile compounds' latent variables that contribute most to the underlying variation in feeding systems. Unsupervised hierarchical clustering analysis was used to show the patterns in the volatile data and is presented as a heatmap. A heatmap can be created by assigning a color to the individual values contained in a matrix (Liu, Zhu, Qiu, & Chen, 2012) and provided an intuitive visualisation of a data table. Consumer acceptance data obtained from the sensory trials were evaluated by one-way ANOVA using cultural differences of perception and feeding systems as primary factors. The post hoc Tukey test was performed to find which feeding systems were significantly different among different cross cultural consumers (Ireland, USA and China). The SPSS V23.0 (IBM Statistics Inc., Armonk NY) was used for Kruskal-Wallis H test and one-way ANOVA. Descriptive data was evaluated by analysis of variance with means separation (SAS version 9.2, Cary, North Carolina, USA). Unscrambler X software, version 10.3 (CAMO ASA, Trondheim, Norway) was used for ANOVA-PSLR (APLSR) analysis of milk powders data in different feeding systems and variance of ODP sensory data to process the raw data accumulated from the +20 test subjects (Fig. 2). The X-matrix was designed as 0/1 design variables for treatment*nationality. The Y-matrix was designed as sensory variables. The optimal number of components in the APLSR models presented was determined to be 4 Principal Components (Fig. 2). PC 1 versus PC 2 is presented; the other PC's did not yield additional information or provide any predictive

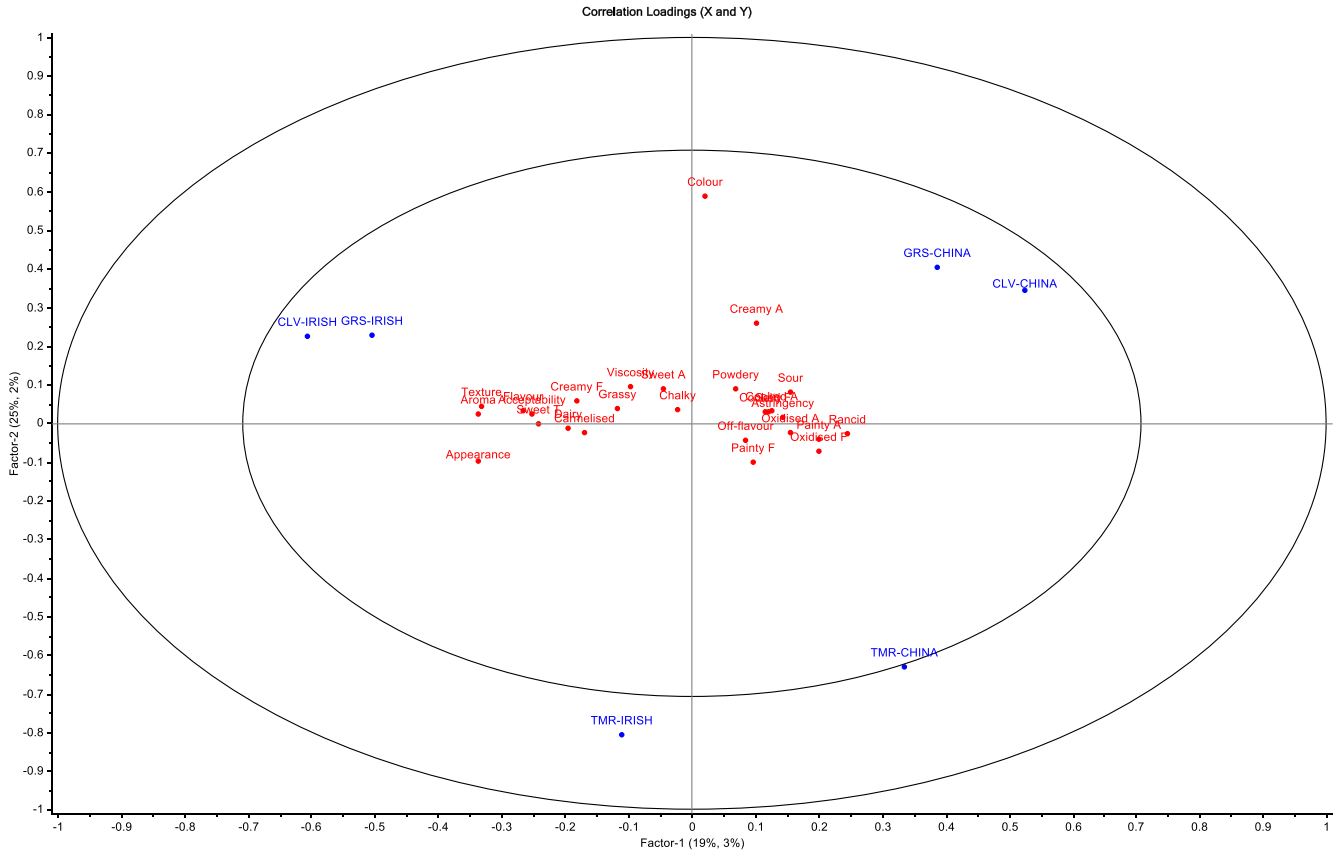

Fig. 2. Optimized Descriptive Profiling. ANOVA-Partial Least Squares regression (APLSR) plot for skim milk powder produced outside on grass (GRS), grass/clover (CLV) or indoors on trial mixed rations (TMR) by as evaluated Chinese (n = 22) and Irish assessors (n = 25). The X-matrix = treatments*nationality (treatment evaluated in China or Ireland). The Y – matrix = sensory variables.

Table 3
The mean scores of consumer acceptance analysis of skim milk powders from Irish, Chinese and USA consumers.

Sensory attributes	I-CLV	I-GRS	I-TMR	C-CLV	C-GRS	C-TMR	U-CLV	U-GRS	U-TMR
Appearance Liking	5.5^{d}	5.4^{d}	5.9^{c}	5.8^{c}	5.8^{c}	5.8^{c}	6.2^{b}	6.2^{b}	6.9^{a}
Aroma Liking	5.4^{b}	5.7^{b}	5.4^{b}	5.4^{b}	5.5^{b}	5.6^{b}	5.6^{b}	5.6^{b}	6^{a}
Overall Liking	5.3^{c}	5.1^{d}	4.4^{e}	5.5^{b}	5.6^{b}	5.6^{b}	5.4^{c}	5.7^{b}	6^{a}
Flavor Liking	5.1^{c}	5.1^{c}	4.4^{d}	5.5^{b}	5.5^{b}	5.4^{b}	5.3^{c}	5.4^{b}	5.8^{a}
Freshness Intensity	2.9^{b}	3^{b}	2.5^{c}	3^{b}	3^{b}	3^{b}	3.2^{b}	3.2^{b}	3.4^{a}
Cooked Flavor Intensity	2.7^{b}	2.7^{b}	3.1^{a}	2.5^{c}	2.5^{c}	2.7^{b}	2.8^{b}	2.7^{b}	2.8^{b}
Mouthfeel/Thickness Liking	5.4^{c}	5.6^{c}	5.1^{c}	1.3^{d}	1.4^{d}	1.6^{d}	6^{a}	5.9^{a}	6.2^{a}
Creaminess Liking	5.4^{c}	4.9^{d}	4.7^{d}	5.3^{c}	5.4^{c}	5.3^{c}	5.6^{b}	5.6^{b}	5.9^{a}
Aftertaste Liking	4.1^{c}	4.1^{c}	3.6^{d}	5.2^{a}	5.3^{a}	5.2^{a}	4.3^{c}	4^{c}	4.6^{b}
Aftertaste Intensity	2.9^{b}	2.6^{c}	3.1^{b}	5.2^{a}	5.5^{a}	5.4^{a}	3^{b}	3.1^{b}	2.8^{b}
Quality	3.1^{a}	3^{a}	2.8^{a}	3.1^{a}	3.3^{a}	3.2^{a}	3^{a}	3.1^{a}	3.3^{a}

Means within a row not sharing a superscript letter are significantly different ($p < 0.05$, post hoc Tukey test)
I denote Irish, C denotes Chinese, and U denotes USA consumers.
CLV = grass/clover, GRS = grass, and TMR = total mixed ration.
Liking attributes were scored on a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely.
Intensity and quality were scored on a 5 point scale where 1 = extremely low and 5 = extremely high.

improvement in the Y-matrix obtained through their examination. To derive significance indicators for the relationships determined in the quantitative APLSR, regression coefficients were analyzed by jack-knifing (Table 4) which is based on cross-validation and stability plots (Martens & Martens, 1999, 2000, 2001a, 2001b). All analyses were performed using the Unscrambler Software, version 10.3 (CAMO ASA, Trondheim, Norway). MetaboAnalyst 4.0 (McGill University, Montreal, QC, Canada) software was used to perform the Variable importance plot (VIP), PLS-DA and Hierarchical clustering analysis (HCA) (Chong et al., 2018).

3. Results and discussion

3.1. Sensory evaluation

3.1.1. Irish, USA and Chinese consumer acceptance testing

The results of the consumer acceptance study of SMP based on different forage types are presented in Fig. 1a,b,c. Significant cross-cultural differences were evident in the perception of the SMP based on diet between the different cultural groups. Chinese consumers rated all SMP dietary types (Fig. 1a,b,c) statistically ($p < 0.05$) higher for "aftertaste liking" and "aftertaste intensity" and lower for "mouthfeel/

Table 4
Regression coefficients from ANOVA-Partial Least Squares regression (APLSR) for Optimized Descriptive Profiling sensory terms of skim milk powders produced from different diets (CLV, GRS and TMR) by Chinese and Irish assessors. Superscript annotation denotes P-values of regression coefficients.

Attributes	Sample					
	CLV-CHINA	GRS-CHINA	TMR-CHINA	CLV-IRISH	GRS-IRISH	TMR-IRISH
Appearance-Colour	0.876854^{**}	1.058541*	-1.43414^{NS}	0.447739^{NS}	0.473134*	-1.94062^{NS}
Sweet Aroma	-0.002^{NS}	0.06389^{NS}	-0.33607^{NS}	0.224464^{NS}	0.199937^{NS}	-0.27293^{NS}
Creamy Aroma	0.65794^{**}	0.672829^{NS}	-0.45454^{**}	-0.07705^{NS}	-0.01318^{NS}	-0.94813^{**}
Cooked Aroma	0.455909*	0.365415^{NS}	0.201013^{NS}	-0.39625^{NS}	-0.31478^{NS}	-0.23528^{NS}
Oxidised Aroma	0.386657^{NS}	0.271352^{NS}	0.36782^{NS}	−0.46719*	-0.38387^{NS}	-0.03825^{NS}
Painty Aroma	0.420726^{**}	0.289852^{NS}	0.42792*	−0.52676**	−0.43409**	-0.01899^{NS}
Chalky Texture	-0.0128^{NS}	0.015315^{NS}	-0.13655^{NS}	0.09811^{NS}	0.086382^{NS}	-0.10038^{NS}
Powdery Texture	0.28357^{NS}	0.265564^{NS}	-0.0709^{NS}	-0.11628^{NS}	-0.07973^{NS}	-0.30647^{NS}
Viscosity	-0.1256^{NS}	-0.03534^{NS}	-0.38976^{NS}	0.331355^{NS}	0.284798^{NS}	-0.20848^{NS}
Sweet Taste	−0.71052*	-0.53002^{**}	-0.51528^{NS}	0.751774^{**}	0.61024^{**}	0.201569^{NS}
Sour Taste	0.484203^{**}	0.409785^{NS}	0.102463^{NS}	-0.34707^{NS}	−0.26855*	-0.34062^{NS}
Salty Taste	0.383679^{NS}	0.306636^{NS}	0.173699^{NS}	-0.33649^{NS}	-0.2676^{NS}	-0.1943^{NS}
Creamy Flavor	-0.41251^{NS}	-0.26668^{NS}	−0.50917*	0.576011*	0.478696*	-0.05463^{NS}
Dairy Sweet Flavor	−0.55868*	−0.42517*	-0.36211^{NS}	0.562512^{**}	0.454328^{**}	0.193685^{NS}
Caramelized Flavor	−0.55975*	−0.4354*	-0.3146^{NS}	0.531564*	0.426648*	0.233449^{NS}
Oxidised Flavor	0.448648^{NS}	0.285196^{NS}	0.578607^{**}	-0.64297^{**}	-0.53534^{**}	0.079697^{NS}
Rancid Butter	0.640691^{**}	0.458645^{NS}	0.563349*	-0.74348^{**}	-0.60874^{**}	-0.10108^{NS}
Painty Flavor	0.110838^{NS}	0.022811^{NS}	0.386808^{NS}	-0.32089^{NS}	-0.27671^{NS}	0.219001^{NS}
Grassy Flavor	-0.22716^{NS}	-0.14647^{NS}	-0.28238^{NS}	0.318521*	0.264788^{NS}	-0.03171^{NS}
Cooked Flavor	0.422567^{NS}	0.338218^{NS}	0.188731^{NS}	-0.36888^{NS}	-0.29319^{NS}	-0.21609^{NS}
Off-Flavor	0.157343^{NS}	0.091127^{NS}	0.248433^{NS}	-0.25574^{NS}	-0.21471^{NS}	0.065148^{NS}
Astringent After-taste	0.389324^{NS}	0.299208^{NS}	0.237368^{NS}	-0.38205^{NS}	−0.30774*	-0.14721^{NS}

Rows differing in asterisk are statistically different, where the significance of regression coefficients *=$p < 0.05$,**=$p < 0.01$,***=$p < 0.001$. NS = not significant. CLV = grass/clover, GRS = grass, and TMR = total mixed ration.

thickness liking". In contrast, USA consumers scored all SMP dietary types significantly higher ($p < 0.05$) for "appearance liking". Moreover, USA consumers also scored "flavor liking", "creaminess liking", "overall liking" and "mouthfeel/thickness liking" statistically higher ($p < 0.05$) in the TMR SMP sample (Fig. 1c), while Irish consumers scored these same attributes significantly lower ($p < 0.05$) in the TMR SMP sample.

The mean scores for hedonic attributes with different diets of SMP for Irish, USA and Chinese consumers are also shown in Table 3. The USA consumers preferred the TMR SMP and found no significant difference ($p < 0.05$) between CLV and GRS SMP, apart from a higher preference ($p < 0.05$) for GRS SMP for "overall liking" and "flavor liking". Irish and USA consumers had similar perceptions of GRS SMP and CLV SMP apart from "appearance liking", "creaminess liking" and "mouthfeel/thickness liking", with USA consumers also scoring the GRS SMP higher ($p < 0.05$) for "overall liking", "flavor liking" and "aftertaste intensity" than the CLV SMP. Irish consumers generally gave higher scores for SMP derived from pasture fed milk ($p < 0.05$) for most attributes, except for "appearance-liking", "cooked flavor intensity" and "aftertaste intensity". No significant differences ($p < 0.05$) were observed between the SMP based on the diet by Chinese consumers, apart from a higher score for "cooked flavor intensity" for the TMR SMP ($p < 0.05$).

Differences in perception between the cultural consumer groups are likely related to product familiarity, as it has been shown to be the significant factor delineating consumer preference tendencies in a cross-cultural context (de Albuquerque et al., 2018; Torrico et al., 2019). It is also thought that the cross-cultural difference in the frame of reference is especially influenced by previous exposure (Kim et al., 2018; Kim, Jombart, Valentin, & Kim, 2015). It is possible that the heat-treatment used in SMP production may also be impacting on sensory perception across the cultural groups. Previous studies have shown that USA and Chinese consumers are more familiar with higher heat-treated dairy products (Song & Kaiser, 2016) and other studies have reported that the nature of the heat treatment used in pasteurization of dairy products has an impact on specific sensory attributes; such as "cooked flavor", "quality", "aftertaste" and "astringency" (Lee, Barbano, & Drake, 2017, Schiano, Harwood, & Drake, 2017). As most retail liquid milk consumption in China is ultra-high temperature (UHT) to overcome transport challenges, extend shelf life and reduce costs (Liem, Bolhuis, Hu, & Keast, 2016), Chinese consumers are likely more familiar with high heat-treated dairy products. Our data shows that USA consumers gave higher scores for "appearance liking", "creaminess liking" and "mouthfeel/thickness liking" in comparison to Irish and Chinese consumers, thus indicating that the extent of temperature of heat-treatment was not negatively impacting on these sensory attributes for USA consumers. However, Irish consumers who are more used to high temperature short time (HTST) pasteurization (72 °C, 15 s) of dairy products (Anon, 2018), may not be as familiar with a high heat-treated (180 °C) SMP type product and this may account for their general lower scores for many of the sensory attributes in comparison to Chinese and USA consumers (Table 3).

USA consumers also scored "appearance liking" significantly higher ($p < 0.05$) for all SMP irrelevant of diet, with the highest score for SMP from TMR. It appears that USA consumers preferred whiter color dairy powders and this may also be due to familiarity as most of the dairy products in the USA are produced from cows fed indoors on TMR rather than pasture. Valverde Pellicer (2007) reported that color is a suitable biomarker to distinguish the appearance of TMR and pasture-fed milk. In previous studies, the yellow color was statistically higher ($P < 0.05$) in milk and butter from pasture-fed cows than from a TMR diet, and directly correlated with tran-β-carotene content (O'Callaghan et al., 2016a; Faulkner et al., 2018). USA consumers also preferred the TMR SMP ($p < 0.05$) for "aroma liking", "overall liking", "flavour liking", "freshness intensity", "creaminess liking" and "aftertaste liking" also indicating that preference is based on familiarity.

Chinese consumers scored higher for "aftertaste liking" and "aftertaste intensity" and lower for "mouthfeel/thickness liking" than the USA and Irish consumers for SMP from all diets. Zhi, Zhao, and Shi (2016) found that a high "aftertaste intensity of thickness and sweetness" is often used as a positive term to describe better quality milk in China and thus the concept of 'aftertaste' may be cultural dependent, because the underlying conceptual elements and words used to describe its features may be dissimilar. Clark, Costello, Drake, and Bodyfelt (2009) reported that good quality milk should have a pleasantly sweet and clean flavor with no distinct aftertaste, and Porubcan and Vickers (2005) found that the aftertaste of milk significantly contributes to the dislike of milk among Western consumers that avoid drinking milk. As the attribute "aftertaste liking" was influenced by diet for Chinese consumers the result may suggest that differences may have more to do with the

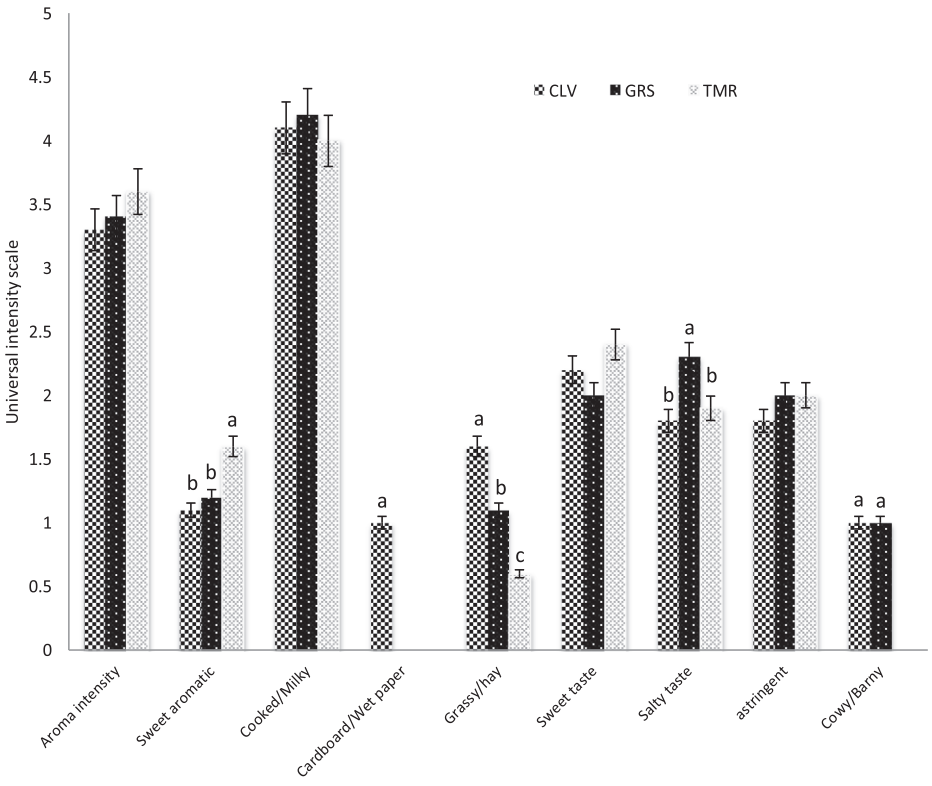

Fig. 3. Descriptive sensory trained attribute intensities of rehydrated skim milk powder derived from different diets by USA assessors (n = 7). The error bars represent mean standard error within replicates. Columns with different letters (a–c) for each attribute are statistically different ($p < 0.05$). Intensities were scored on a 0 to 15-point universal scale, most dairy powder flavors fall between 0 and 4 on this scale (Drake et al., 2009). CLV = grass/clover, GRS = grass, and TMR = total mixed ration.

verbalisation of sensory perception and linguistic representation, rather than due to the Western definition of the term. Difficulties associated with transmitting sensory concepts among countries have been previously described with some words difficult to categorise with ambiguous cultural meanings (Son et al., 2014).

3.1.2. Optimized descriptive profiling – Irish and Chinese trained assessors

The ODP evaluation of SMP from different diets is shown in the APLSR plot, Fig. 2. The SMP samples were subdivided into three distinct cluster groups based on the evaluations by the Irish and Chinese assessors. No significant sensory differences ($p < 0.05$) were evident between SMP derived from GRS and CLV diets for both Chinese and Irish assessors, while both groups discerned that TMR SMP was significantly ($p \leq 0.05$) different to SMP from GRS and CLV diets.

The significance (p-value) of regression coefficients for the ODP attributes described by the Irish and Chinese assessors for SMP from different diets (CLV, GRS and TMR) are illustrated in Table 4. The CLV SMP and GRS SMP samples were rated significantly higher for "creamy flavor" ($p < 0.05$), "dairy sweet flavor" ($p < 0.01$), "sweet taste" ($p < 0.01$) and "caramelized flavor" ($p < 0.05$) by Irish assessors, which again may be linked to familiarity with pasture derived dairy products. It is interesting that the Irish assessors significantly scored "sweet taste" ($p < 0.05$) higher for both CLV and GRS SMP and that Chinese assessors found a negative perception of "sweet taste" for both CLV ($p < 0.05$) and GRS ($p < 0.01$) SMP, as previously Chung, McDaniel, and Lundahl (2010) determined that the optimal sweetness level for a sensory evaluated sports-drink was lower for American than Asian consumers, perhaps displaying a higher propensity for sweet beverages in the latter group.

The Chinese assessors scored the color ("appearance-color) of GRS ($p < 0.05$) and CLV SMP ($p < 0.01$) significantly higher than TMR SMP, while the Irish assessors only rated a significantly higher score for the color of GRS SMP ($p < 0.05$) (but did give a numerically greater score for CLV than TMR SMP) (Table 4). Chinese assessors scored "creamy aroma" ($p < 0.01$), "sour taste" ($p < 0.01$) and "cooked aroma" significantly ($p < 0.05$) higher for the CLV SMP sample, and both Irish and Chinese assessors had a significantly ($p < 0.01$) negative perception of "creamy aroma" in TMR SMP. Irish assessors had a significant negative score for "sour taste" ($p < 0.05$) for GRS SMP. "Oxidised aroma" was statistically negatively ($p < 0.05$) associated with the CLV SMP sample by Irish assessors, who also found that "oxidised flavor" and "rancid butter" were negatively associated with the CLV ($p < 0.01$) and GRS ($p < 0.01$) SMP. Irish assessors also found a negative association of "painty aroma" with both CLV and GRS ($p < 0.01$) SMP, indicating further preferences for CLV and GRS over TMR SMP. Chinese assessors scored "painty aroma" and "rancid butter" higher in CLV ($p < 0.01$) and TMR ($p < 0.05$) and had a significantly higher score ($p < 0.01$) for "oxidised flavour" in the TMR SMP than Irish assessors."Astringent after-taste" in GRS SMP was significantly ($p < 0.05$) negatively correlated by Irish assessors and who also significantly ($p < 0.05$) correlated "grassy flavor" to only the CLV SMP. This is in partial agreement with Croissant et al. (2007) who found greater intensities of grassy and cowy/barny flavors in pasture based milks compared with TMR milks when evaluated at 15 °C using trained panelists. Similarly, the present results are in partial agreement with Villeneuve et al. (2013), who using a sensory ranking test, found that percentage of assessors ranking for the intensity of grassy (grass, leafy vegetable, and plant) flavors was higher for milk from cows fed pasture compared with hay and silage. The other sensory attributes ("sweet aroma", "chalky texture", "powdery texture", "viscosity", "salty taste", "painty flavor", "cooked flavor" and "off-flavor") were not significantly different between Chinese and Irish assessors.

3.1.3. Descriptive analysis by USA trained panelists

The CLV and GRS SMP samples scored statistically higher ($P < 0.05$) for "grassy/hay" (highest in CLV SMP samples) and the GRS SMP sample scored statistically highest ($P < 0.05$) for "salty taste" (Fig. 3). In contrast, the TMR SMP scored significantly higher ($p < 0.05$) for "sweet aromatic". "Cardboard/wet paper" was only detected in the CLV SMP. The panel also commented on the fact that the CLV SMP and GRS SMP samples had a pronounced "cowy/barny" attribute which was absent in

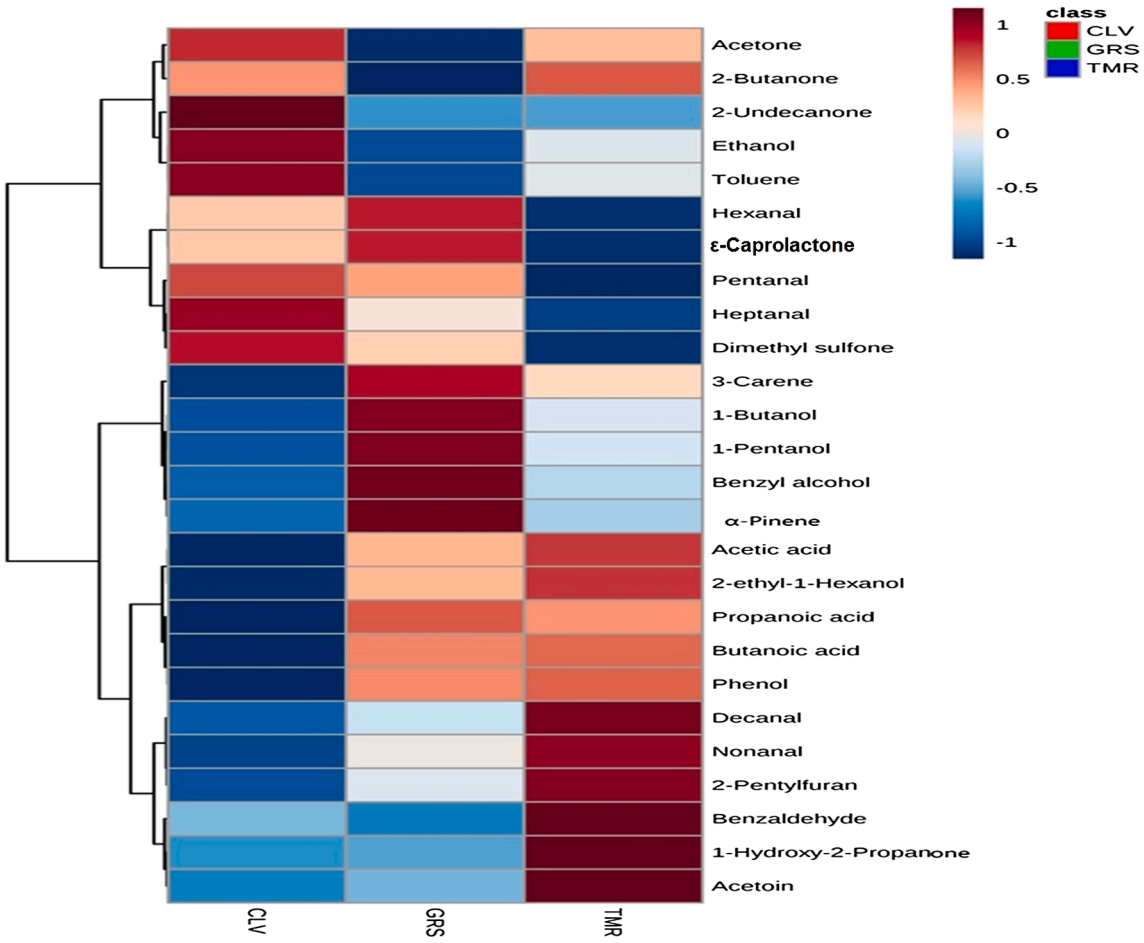

Fig. 4. Hierarchical clustering analysis (heatmap) of volatile compounds derived from different feeding systems; grass (GRS), grass/clover (CLV) and total mixed ration (TMR). The degree of positive and negative correlation of skim milk powder is indicated by + 1 (red) to −1 (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the TMR SMP sample. As stated previously a "barnyard" aroma in milk was associated with pasture diets, and found to be highest in CLV milk (Faulkner et al., 2018). Grassy and hay flavors have been previously documented by trained US panelists in dairy products from cows fed pasture based diets (Croissant et al., 2007; Drake et al., 2005; Drake, Miracle, & Wright, 2009). The USA panelists did not find any signficant differences in "aroma intensity", "cooked/milky", "sweet taste" or "astringent" between the CLV, GRS and TMR SMP.

3.2. Volatile analysis

Diet was shown to have a significant effect ($p < 0.005$) on the volatile compounds of SMP (Fig. 4). In total, 26 volatile compounds were identified in these SMP samples (Table 5). The TMR SMP samples had the greatest abundance of acetic acid, nonanal, decanal and acetoin ($p < 0.01$), and 1-hydroxy-2-propanone (hydroxyacetone) and propanoic acid ($p < 0.05$). The CLV SMP had the greatest abundance of ethanol, dimethyl sulfone ($p < 0.05$), pentanal and heptanal ($p < 0.01$). No significant differences ($p < 0.05$) were observed between diets for butanoic acid, 1-butanol, 1-pentanol, phenol, 2-ethyl-1-hexanol, benzyl alcohol, hexanal, benzaldehyde, 2-pentylfuran, acetone, 2-butanone, ε-caprolactone, 2-undecanone, α-pinene, 3-carene and toluene. Variable importance coefficients (VIP) scores highlight those volatiles contributing most to the observed discrimination (Fig. 5). Volatiles with a VIP ≥ 0.80 discriminating TMR SMP were predominantly metabolic carbohydrate products such as acetoin and acetic acid, which were highest in TMR SMP and lowest in CLV SMP. Moreover, the GRS and CLV SMP samples had greater levels of dimethyl sulfone, heptanal, pentanal and 2-undecanone arising from protein metabolism and lipid oxidation, respectively. Both the GRS SMP and CLV SMP samples are more closely associated, with a distinct separation from the TMR SMP samples on PLS-DA plot (Supplementary Fig. 1). The APLSR plot (Supplementary Fig. 2) of the average volatiles in the SMP samples from each diet, clearly separates volatile components in the SMP samples on a dietary basis. It is worth noting that volatile discrimination in this study is based on abundance levels alone and does not take into account differences in odor activity between the volatiles.

Short chain carboxylic acids (SCFFA) are derived from carbohydrate metabolism, lipolysis or amino acid metabolism but may also be transferred directly from forage due to their volatility (Kilcawley et al., 2018) and contribute to the sour and cheese flavor of milk (Parker, Elmore, & Methven, 2014). Acetic and propanoic acid were statistically higher in SMP from the TMR diet in comparison to SMP from GRS and CLV (Table 5). This is most likely due to the higher carbohydrate content of TMR which is metabolised in the rumen to these short-chain FFA (Coppa et al., 2011). The CLV SMP samples were perceived as having the highest score for both "cardboard/wet paper" and "cowy/barny" aftertaste by DA analysis by USA panelists (Fig. 3). Pentanal and heptanal are primary products of lipid oxidation and associated with "cardboard" flavor (Whitson, Miracle, & Drake, 2010) and were significantly higher in the CLV SMP than the SMP derived from either GRS or TMR. Park and Drake (2016) found that "cardboard" flavor was inversely proportional to a lower "sweet aromatic" flavor in SMP, it worth nothing that "cardboard/wet paper flavor" as detected by USA panelists in the CLV SMP also had

Table 5
Volatile compounds identified by head space solid phase micro-extraction gas chromatography mass spectrometry in skim milk powder produced from different diets [grass (GRS), grass/clover (CLV) and total mixed rations (TMR)]; values indicate abundance values for each compound.

Volatile compound	CAS no.[a]	LRI [a]	CLV	GRS	TMR	P-value
Acid						
Acetic acid	64-19-7	687	4.47E+04	7.55E+04	9.07E+04	***
Propanoic acid	79-09-4	777	9.35E+03	1.59E+04	1.60E+04	*
Butanoic acid	107-92-6	859	1.16E+05	1.60E+05	1.73E+05	NS
Alcohol						
Ethanol	64-17-5	512	9.12E+04	7.68E+04	8.27E+04	*
1-Butanol	71-36-3	713	3.78E+04	3.93E+04	3.95E+04	NS
1-Pentanol	71-41-0	813	4.47E+03	6.65E+03	5.07E+03	NS
Phenol	108-95-2	1092	1.03E+04	1.16E+04	1.17E+04	NS
2-Ethyl-1-hexanol	104-76-7	1073	3.96E+05	4.32E+05	4.43E+05	NS
Benzyl alcohol	100-51-6	1119	1.17E+04	1.22E+04	1.20E+04	NS
Aldehyde						
Pentanal	110-62-3	734	1.10E+04	9.77E+03	6.51E+03	***
Hexanal	66-25-1	837	4.38E+04	4.39E+04	4.21E+04	NS
Heptanal	111-71-7	941	2.06E+04	1.63E+04	1.25E+04	***
Benzaldehyde	100-52-7	1027	2.01E+04	1.88E+04	2.65E+04	NS
Nonanal	124-19-6	1146	5.98E+04	7.04E+04	8.24E+04	***
Decanal	112-31-2	1250	8.12E+03	9.08E+03	1.09E+04	***
Furan						
2-Pentylfuran	3777-69-3	1043	1.89E+03	2.51E+03	3.52E+03	NS
Ketone						
Acetone	67-64-1	535	1.64E+05	1.17E+05	2.00E+05	NS
2-Butanone	78-93-3	636	1.94E+04	1.56E+04	2.43E+04	NS
1-Hydroxy-2-Propanone	116-09-6	733	2.17E+04	2.17E+04	4.07E+04	*
Acetoin	513-86-0	776	1.84E+03	3.54E+03	9.00E+04	***
ε-caprolactone	502-44-3	1276	2.09E+04	2.21E+04	1.92E+04	NS
2-Undecanone	112-12-9	1344	7.26E+03	2.83E+03	3.13E+03	NS
Sulfur compound						
Dimethyl sulfone	67-71-0	1051	2.07E+05	1.81E+05	1.23E+05	*
Terpene						
α-Pinene	80-56-8	952	2.77E+04	3.32E+04	2.99E+04	NS
3-Carene	13466-78-9	1031	2.69E+04	3.14E+04	2.93E+04	NS
Phenyl						
Toluene	108-88-3	794	4.50E+03	4.51E+03	6.73E+03	NS

Kruskal-Wallis Ranking test statistical analysis:* and *** denote significant differences at $P < 0.05$ and $P < 0.001$, respectively.
[a] CAS no. = Chemical Abstracts Service number. LRI = linear retention index.

the lowest intensity of "sweet aromatic" aroma (Fig. 3). As stated, nonanal and decanal are also products of lipid-oxidation and were statistically higher ($P < 0.05$) in SMP derived from TMR, Chinese consumers also found that "oxidised flavor" was statistically higher for SMP TMR. Boltar, Majhenič, Jarni, Jug, and Krali (2015) found nonanal and decanal were significantly higher in winter Nanos cheese and suggested that these compounds are not present in pasture-fed products. Park, Bastian, Farkas, and Drake (2013) found that nonanal and decanal had been attributed to "off-flavor (cardboard, fatty)" during UHT processing.

In ruminants, ethanol is oxidized to acetaldehyde by alcohol dehydrogenase and has been shown to be directly transferred from forage (maize silage) to milk (Randby, Selmer-Olsen, & Baevre, 1999). Toso, Procida, and Stefanon (2002) also demonstrated that ethanol had a significant role in milk derived from preserved forages and was a major discriminator. Our study indicated that ethanol was in greater abundance in SMP from CLV, but it must be noted that ethanol is not very odour active (Kilcawley et al., 2018). The ketone content of bovine milk has also been shown to be significantly affected by diet (Stefanon & Procida, 2004), with ketones derived from carbohydrate metabolism higher in the feed with greater levels of carbohydrate, such as in TMR. In this study, hydroxyacetone and acetoin primarily products of carbohydrate metabolism, were positively correlated with TMR samples (Supplementary Fig. 2). Each feeding system had a significant effect on the concentrations of acetoin (TMR > GRS > CLV) and it was found to be the most discriminatory compound based on abundance levels between the diets by VIP (Fig. 5), as determined by PLS-DA. As we did not undertake olfactory analysis or quantification we cannot assess its sensory impact but acetoin has an intermediate odour threshold and has the potential to influence sensory perception. Volatile sulfur compounds are also potentially crucial due to their high odor intensities and are derived from methionine and cysteine by rumen microbes (Faulkner et al., 2018). Dimethyl sulfone was most abundant in the CLV-SMP and at lowest abundance in the TMR-SMP and this is in agreement with other studies which found higher levels of dimethyl sulfone in milk and cheese from cows on pasture diets (Coppa et al., 2011; Faulkner et al., 2018; Villeneuve et al., 2013). *p*-Cresol is a rumen metabolite associated with the degradation of β-carotene (Agabriel et al., 2004) and has been found at higher levels in pasture-derived dairy products (Faulkner et al., 2018; O'Callaghan et al., 2018). Lopez and Lindsay (1993) noted that the *p*-cresol has a "barn-yard" like odor and is responsible for "cowy flavor". Although *p*-cresol was not detected in SMP in this study, USA panels (Fig. 3) identified a "barnyard flavor" in SMP from GRS and CLV which was not present in TMR SMP. Thus, it appears that *p*-cresol may be present below levels of detection by HS-SPME GCMS, but above its odor threshold.

4. Conclusion

This study investigated the sensory perception of SMP produced by three separate feedings systems in a cross-cultural context between Irish, USA and Chinese consumers, trained Irish and Chinese assessors, and a trained USA sensory panel. Significant cross-cultural differences were evident in the perception of the SMP based on diet between the different cultural groups, and diet also influenced the volatile profile of SMP.

Chinese consumers could not discern a difference between the CLV, GRS and TMR SMP, but rated "aftertaste liking", "aftertaste intensity" and "mouthfeel/thickness liking" differently than USA and Irish consumers. In general, USA consumers had preference for TMR SMP, but did not find many differences between CLV and GRS SMP. Irish consumers had lower scores for many attributes than both Chinese and USA

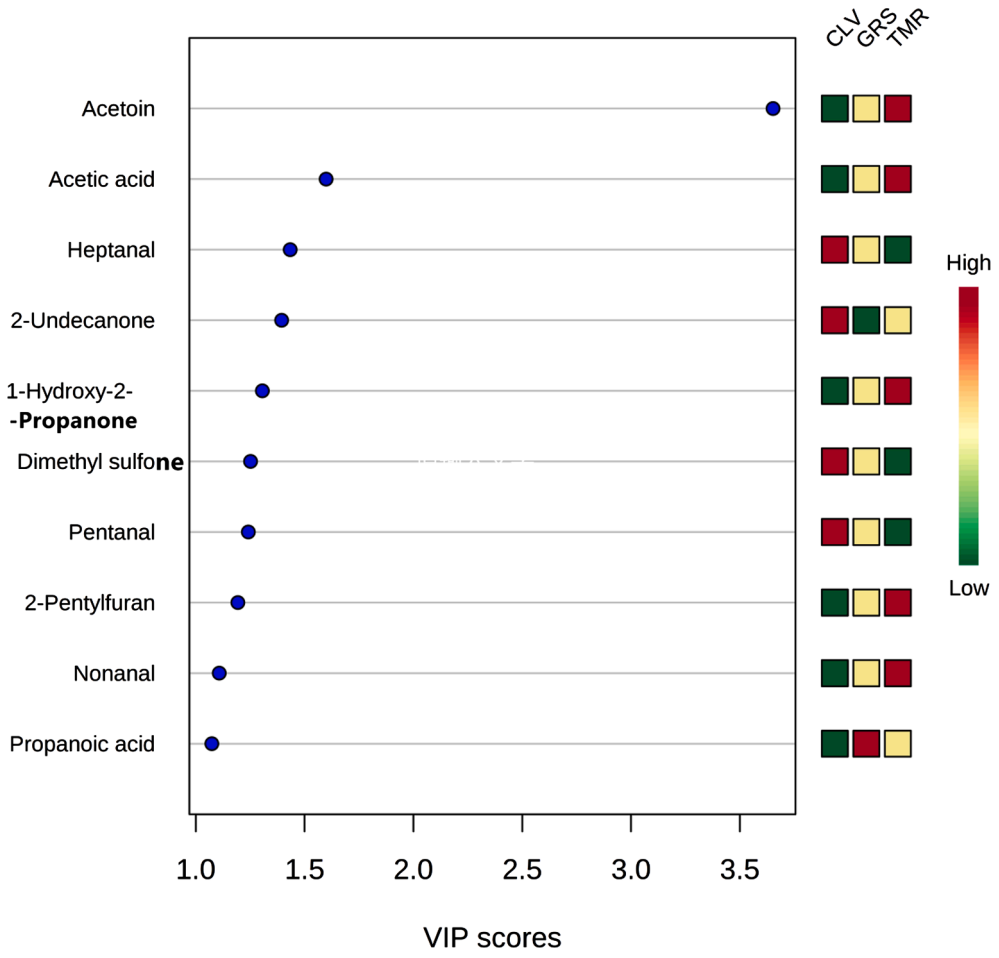

Fig. 5. Variable Importance in Projection (VIP) plot shows the relevance of the most significant volatiles responsible for the differentiation between the skim milk powder produced from different feeding regimes (CLV, GRS and TMR) samples, as determined by Partial Least Squares- Discriminate Analysis (PLS-DA). CLV = grass/clover, GRS = grass, and TMR = total mixed ration.

consumers, but generally preferred CLV and GRS SMP than TMR SMP. ODP analysis by trained Chinese and Irish assessors found they could more easily discern that TMR SMP was different to CLV and GRS SMP. In general Irish assessors rated many positive attributes higher, and negative attributes lower in CLV and GRS SMP than in TMR SMP, although they only gave a negative association for "creamy aroma" in TMR SMP. Chinese assessors did not find any significant differences in 11 of the 22 attributes evaluated, but rated TMR negatively for "creamy aroma" and "creamy flavour" and positively for "painty aroma", "oxidised flavour" and "rancid butter". Chinese assessors did find a positive association with "creamy aroma", "cooked aroma", "painty aroma", "sour taste", and "rancid butter" for CLV SMP in comparison to GRS SMP, and a negative association for "sweet taste", "dairy sweet taste", and "carmelized flavour" for both CLV and GRS SMP in comparison to TMR SMP. Trained descriptive USA panelists found that TMR SMP was more "sweet aromatic", with less "grassy/hay" attributes than CLV or GRS SMP and that GRS SMP had more "salty taste" than CLV or TMR SMP. The CLV SMP had a "cardboard/wet paper" attribute and both CLV and GRS SMP had a "cowy/barny" attribute which were absent in TMR SMP. Ten volatile compounds differed statistically based on diet, with acetoin derived from carbohydrate metabolism having the greatest impact based on abundance levels, and was significantly higher in TMR SMP than in CLV or GRS SMP.

This study has highlighted significant cross cultural sensory differences in SMP produced from pasture (CLV and GRS) and non-pasture (TMR) feeding systems, which are likely applicable to other dairy products produced from similar feeding regimes. It also highlighted differences between consumers and trained panellists, that were most apparent between Chinese consumers and Chinese trained assessors. Some of the main volatiles most likely responsible for these differences were also identified. The study has highlighted the importance of product familiarity, as USA and Irish consumers, trained assessors and trained panelists preferences were essentially aligned with their familiarity to dairy products from non-pasture and pasture feeding regimes, respectively. Chinese consumers were less discerning in relation to diet, but both Chinese consumers and trained assessors scored many attributes quite differently than their USA or Irish counterparts, likely reflecting a lack of familiarity with dairy products in general.

Author contributions

Zeng Cheng was the main author and undertook most of the experimental work. Kieran Kilcawley is Zeng Cheng's main supervisor and was actively involved in experimental design, and editing the manuscript through all stages. Dr Maurice O'Sullivan is a co-academic supervisor for Zeng Cheng, and collaborated in undertaking the sensory experimental work in Ireland and provided advice in relation to the sensory work in China, and also edited the final draft of the manuscript. Prof Joe Kerry is the also an academic supervisor for Zeng Chen and helped editing the final version of the manuscript. Prof MaryAnne Drake collaborated with the sensory analysis is the USA, aided with experimental design, statistical analysis and editing the final draft of the manuscript. Prof Song Miao and Dr Deng Kaibo collaborated with the sensory analysis in China.

CRediT authorship contribution statement

Zeng Cheng: Data curation, Investigation, Writing - original draft. **Maurice O'Sullivan:** Supervision, Methodology, Validation, Writing - review & editing. **Joe Kerry:** Supervision. **MaryAnne Drake:** Data curation, Formal Analysis, Investigation, Validation, Writing - review & editing. **Song Miao:** Resources. **Deng Kaibo:** Data curation, Investigation. **Kieran Kilcawley:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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Comparison of Automated Extraction Techniques for Volatile Analysis of Whole Milk Powder

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Abstract: Volatile profiling of whole milk powder is valuable for obtaining information on product quality, adulteration, legislation, shelf life, and aroma. For routine analysis, automated solventless volatile extraction techniques are favored due their simplicity and versatility, however no single extraction technique can provide a complete volatile profile due to inherent chemical bias. This study was undertaken to compare and contrast the performance of headspace solid phase microextraction, thermal desorption, and HiSorb (a sorptive extraction technique in both headspace and direct immersion modes) for the volatile analysis of whole milk powder by gas chromatography mass spectrometry. Overall, 85 unique volatiles were recovered and identified, with 80 extracted and identified using a non-polar gas chromatography column, compared to 54 extracted, and identified using a polar gas chromatography column. The impact of salting out was minimal in comparison to gas chromatography column polarity and the differences between the extraction techniques. HiSorb extracted the most and greatest abundance of volatiles, but was heavily influenced by the number and volume of lactones extracted in comparison to the other techniques. HiSorb extracted significantly more volatiles by direct immersion than by headspace. The differences in volatile selectivity was evident between the techniques and highlights the importance of using multiple extraction techniques in order to obtain a more complete volatile profile. This study provides valuable information on the volatile composition of whole milk powder and on differences between extraction techniques under different conditions, which can be extrapolated to other food and beverages.

Keywords: whole milk powder; automated volatile extraction; gas chromatography mass spectrometry; HiSorb; headspace solid phase microextraction; thermal desorption

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1. Introduction

The global production of whole milk powder (WMP) was 10.8 million tons in 2019 and is anticipated to reach 13.2 million tons by 2024 [1]. It remains a considerable export product for Ireland with 57,000 tons exported in 2019 [2]. Dairy powders such as WMP have unique flavor characteristics that are heavily influenced by fat content and fat distribution [3], but are also very susceptible to lipid oxidation [4]. Many studies on the volatile properties of dairy products have only evaluated single extraction techniques. However, as all extraction techniques have inherent bias towards certain volatiles based upon the properties of the volatile organic compounds (VOCs), their affinity to the sample matrix, and the properties

and parameters of the extraction technique [5], it is therefore useful to evaluate a wider range of extraction techniques in order to get the best possible representative volatile profile of a sample.

Arguably the most widely used volatile extraction technique to date is solid phase microextraction (SPME), mainly due to its versatility, ease of use (as it is fully automatable), the wide range of coating materials available (single, dual, or multiple phases in different thicknesses), and its general robustness. It can be used as a direct immersion (DI) technique or, most commonly, as a headspace (HS) technique. Headspace solid phase microextraction (HS-SPME) is a static HS technique that has been extensively applied to analyze VOCs in dairy products, with the divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber finding the greatest use, due to its potential ability to capture a broader range of VOCs owing to the inherent properties of each phase [6–8]. However, the relatively limited surface capacity of the fiber can result in competition between analytes for adsorption/absorbtion sites and displacement resulting in increased bias for certain VOCs [9]. Thermal desorption (TD) is a well-established dynamic extraction technique, where an inert carrier gas strips the volatiles from a sample where they are subsequently trapped in a sorbent packed tube with absorbent/adsorbent material [10,11]. The main advantages are the wide range of sorbent phases available and the large capacity of sorbent phase. However, managing moisture can be problematic, and this may be why its use in dairy applications is limited [12,13]. Tenax (TEN) is typically the most widely used sorbent material in TD because of its affinity for VOCs with a very wide range of boiling points between 60 °C and 300 °C [14]. Recently, another passive sorbent extraction technique was developed called HiSorb™ (Markes International Ltd., Llantrisant, UK). It is somewhat similar to stir bar sorptive extraction (SBSE) [15] and can also be performed as a headspace (HS) or as a direct immersion (DI) technique. With HiSorb to date, a single sorbent phase polydimethylsiloxane (PDMS) is coated in a specially designed probe that can be either exposed to a HS above a sample or directly immersed in a liquid sample under controlled conditions. After exposure, the probe is placed in an empty sorbent tube and treated in a similar manner to a TD sorbent tube, where it is desorbed.

In terms of sample preparation, "salting out" is a useful practice to potentially increase the extraction efficiency of certain volatile analytes. Salt, usually sodium chloride (NaCl), is added to the sample, which reduces the solubility of hydrophobic compounds, resulting in decreased water availability and thus, in theory, making polar and low molecular weight VOCs easier to extract [16]. The polarity of the gas chromatography (GC) column is also an important factor in relation to the separation of individual VOCs. The most common types are polar and non-polar phases, both of which offer better separation and resolution for specific chemical classes [17], with non-polar phases having greater stability. Therefore, in order to obtain the best possible volatile profile, it is also useful to assess both polar and non-polar GC columns.

In this study, we compared the ability of four automated volatile extraction techniques (HS-SPME, TD, and HiSorb as HS (HS-HiSorb) and as DI (DI-HiSorb)) for their ability to profile volatile compounds in WMP using gas chromatography mass spectrometry (GC-MS). Each extraction technique was assessed with or without salting out and using both a polar and non-polar GC column.

2. Materials and Methods

2.1. Preparation of Whole Milk Powder

Raw milk was produced from 54 Friesian cows at the Teagasc Moorepark dairy farm, Fermoy Co., Cork, Ireland. The milk was pre-heated to 50 °C in an APV plate heat exchanger (SPX Flow Technology, Crawley, West Sussex, UK), separated by a centrifugal disk separator, and pasteurized at 72 °C for 15 s. The pasteurized milk was subsequently preheated to 78 °C and evaporated in a Niro three-effect falling film evaporator (GEA Niro A/S, Soeborg, Denmark) at sequential effect temperatures of 73 °C, 64 °C, and 55 °C. Concentrate feed was introduced to a Niro Tall-Form Anhydro three-stage spray

dryer (GEA Niro A/S, Soeborg, Denmark) (air inlet temperature = 180 °C and air outlet temperature = 85 °C) at approximately 43% total solids (TS) with a centrifugal atomizer (GEA Niro A/S, Soeborg, Denmark) at Moorepark Technology Ltd. (Fermoy Co., Cork, Ireland). Primary and secondary fluidized beds were maintained at 74 °C and 24 °C, respectively. Fines were returned to the cyclone to the top of the spray dryer. WMP samples were stored at room temperature in sealed 900 g aluminum vacuum cans until analysis.

WMP samples were dissolved at 10% solids (w/v) using ultra-pure deionized water and stored at 4 °C overnight to ensure solubility, without overhead lights to prevent light-induced off-flavor formation. Each extraction technique was assessed with or without salting out. NaCl (0.75 g) (Merck, Co., Wicklow, Ireland) was added to 5 mL of the 10% w/v WMP sample, equivalent to 15% NaCl w/v. This was mixed until soluble (~30 min).

2.2. Internal and External Standard Preparation

To monitor the performance of the GC-MS operating conditions, an external standard (ES) solution was added at the start and end of each GC-MS sample run. The ES was comprised of 1-butanol, dimethyl disulfide, butyl acetate, cyclohexanone, and benzaldehyde (Merck, Ireland) at 10 mg L^{-1} with 2-phenyl-D5-ethanol (Merck, Arklow, Co., Wicklow, Ireland) added at 5 mg L^{-1} in ultra-pure water. For the HS-SPME technique, 10 µL of ES was added to the sample in a 20 mL amber La-Pha-Pack HS vial with magnetic screw caps and a silicone/polytetraflurorethylene septa (Apex Scientific Ltd., Maynooth, Ireland); see details in Section 2.3.1. The ES (10 µL) was also added to the TD tube containing the sample extract for both TD and HiSorb (HS-HiSorb and DI-HiSorb), the details of which are described in Sections 2.3.2 and 2.3.3. To monitor the performance of each extraction procedure, an internal standard (IS) of 2-phenyl-D5-ethanol and 4-methyl-2-pentanol (Merck, Arklow, Co., Wicklow, Ireland) at 20 mg L^{-1} in ultra-pure water, was added (50 µL) to each WMP sample prior to extraction.

2.3. Extraction Procedures

The following codes were used to describe each extraction technique with and without salting out for both polar and non-polar GC columns (Table 1).

Table 1. Details and codes used to describe each extraction technique with and without salting out evaluated.

Code	Description
HS-SPME S	Head space solid phase microextraction with salting out
HS-SPME NS	Head space solid phase microextraction without salting out
TD S	Thermal desorption with salting out
TD NS	Thermal desorption without salting out
DI-HiSorb S	Direct Immersion HiSorb with salting out
DI-HiSorb NS	Direct Immersion HiSorb without salting out
HS-HiSorb S	Head space HiSorb with salting out
HS-HiSorb NS	Head space HiSorb without salting out

An extraction temperature of 40 °C was used for each technique based on previous experience and to ensure sufficient VOC extraction without creating additional VOC due to Maillard reactions or caramelization during the extraction process. The extraction times varied between techniques based on specific aspects of each technique and on previous experience. An equilibration step was necessary for the HS-SPME to maximize the VOC concentration in the HS prior to extraction.

2.3.1. Head-Space Solid Phase Microextraction (HS-SPME)

The WMP solutions (5 mL) were added to a 20 mL amber La-Pha-Pack vial (as described in Section 2.2) and equilibrated to 40 °C for 10 min, with pulsed agitation of 5 s at 500 rpm using an Agilent GC 80 Autosampler (Agilent Technologies Ireland Ltd., Cork, Ireland). Each sample was pre-incubated at 40 °C with pulsed agitation for 10 min. A single SPME 50/30 µm (DVB/CAR/PDMS) fiber (Agilent Technologies Ireland Ltd., Cork, Ireland) was exposed to the headspace above the samples in the vial for 20 min at a depth of 1 cm at 40 °C. Following extraction, the SPME fiber was retracted and injected into the gas chromatograph inlet and desorbed for 3 min at 250 °C in splitless mode. The fiber was cleaned in a bakeout conditioning station (Agilent Technologies Ireland Ltd., Cork, Ireland), between each sample injection, at 270 °C with a nitrogen flow of 6 mL min^{-1}, and blanks were conducted after every triplicate sample to ensure no carryover occurred. A Merlin microseal (Agilent Technologies Ireland Ltd., Cork, Ireland) was used to minimize fiber wear. Each sample was analyzed in triplicate.

2.3.2. Thermal Desorption Extraction

A micro-chamber/thermal extractor (Markes International Ltd., Llantrisant, UK) was used for dynamic headspace extraction using industry standard TD tubes packed with Tenax/Carbograph (TEN/CAR) (Markes International Ltd., Llantrisant, UK). The analysis was undertaken in triplicate and the TEN/CAR tubes were preconditioned at 280 °C for 1 hr prior to sampling using a TC-20 (Markers International Ltd., Llantrisant, UK). A Unity 2 thermal desorption unit (Markes International Ltd., Llantrisant, UK) was used to concentrate the volatiles and remove excess moisture. A heated transfer line was used to automatically transfer the volatiles from the Unity 2 to the GC. The WMP solution (5 mL), containing the IS, was added to an inert stainless steel microchamber pot and extracted in the micro-chamber at 40 °C at 50 mL min^{-1} in nitrogen for 20 min. Each sorbent tube was desorbed in the Unity 2 thermal desorption unit with a materials emission focusing trap (Markes International Ltd., Llantrisant, UK). The sample tubes were initially pre-purged for 2 min with a 1:20 split, followed by a two-stage desorption. In the first stage, the tubes were ramped to 110 °C with a 1:10 split for 10 min, then heated to 280 °C for 10 min without a split. The cold trap was set at 30 °C, with a trap flow of 50 mL min^{-1}. After tube desorption, a pre-trap fire purge was performed for 2 min, before heating the trap to 300 °C at 100 °C s^{-1} for 5 min without a split. The transfer line was held at 160 °C. Each sample was analyzed in triplicate.

2.3.3. Headspace and Direct Immersion Hi-Sorb Extraction

The WMP samples (5 mL) were pipetted into a 20 mL amber La-Pha-Pack vial (Apex Scientific Ltd, Maynooth, Co., Kildare, Ireland) with a HiSorb-P1 inert PDMS probe assembly (Markes International Ltd., Llantrisant, UK) for both HS-HiSorb and for DI-HiSorb. For DI-HiSorb, the HiSorb probe was directly immersed in the liquid WMP sample and sealed. For HS-HiSorb, the probe was placed at a fixed depth of 1 cm above the sample in the vial (care was taken to ensure that the probe remained dry) and sealed. The vials were added to the HiSorb Agitator (Markes International Ltd., Llantrisant, UK) and agitated at 250 rpm for 120 min at 40 °C for the DI-HiSorb. The vials were added to the HiSorb agitator at 250 rpm for 180 min at 40 °C for the HS-HiSorb. The HiSorb probes were rinsed with deionized water and gently dried with a lint-free tissue prior to insertion into a clean, empty TD tube (Markes International Ltd., Llantrisant, UK), which were end capped using brass long-term storage caps (Markes International Ltd., Llantrisant, UK). The TD tubes were then evaluated in an identical manner to that described for the TD extraction. Each HiSorb probe was preconditioned at 280 °C for 1 h between samples using a U-CTE micro-chamber/thermal extractor (Markes International Ltd., Llantrisant, UK).

2.4. GC-MS Analysis

The GC-MS system was an Agilent 7890A GC and Agilent 5977B MSD (Agilent Technologies Ireland Ltd., Cork, Ireland). The analysis was undertaken using both a non-polar GC column DB5-MS (60 m × 0.25 mm × 0.25 µm) and a polar GC column HP-Innowax (60 m × 0.25 mm × 0.5 µm) (Agilent Technologies Ireland Ltd., Cork, Ireland). The GC conditions for the non-polar DB5-MS column were as follows: the injector temperature was set at 250 °C, while the column was initially at 35 °C, then increased to 230 °C at 6.5 °C min^{-1}, 320 °C at 15 °C min^{-1}, before being held for 5 min, yielding a total run time of 41 min. The carrier gas helium was held at a constant pressure of 23 psi. The GC conditions for the polar HP-Innowax column were as follows: the injector temperature was set at 250 °C, while the column was initially at 40 °C for 5 min, then increased to 230 °C at 5 °C min^{-1}, before being held for 10 min, yielding a total run time of 59 min. The carrier gas helium was held at a constant pressure of 23 psi.

The ion source temperature was 220 °C and the interface temperature was set at 260 °C. The mass spectrometer was in electronic ionization (70 v) mode with the mass range scanned between 35 and 250 amu. The analysis was undertaken using MassHunter Qualitative Analysis software (Agilent Technologies, Palo Alto, CA, USA) with target and qualifier ions and linear retention indices for each compound compared to an in-house library based on mass spectra obtained from NIST 2014 mass spectral library MS searching (v.2.3, Gaithersburg, MD, USA) and an in-house library created using authentic compounds with target and qualifier ions and linear retention indices for each compound using the Kovats index. Spectral deconvolution was also performed to confirm identification of compounds using the automated mass spectral deconvolution and identification system (AMDIS). Batch processing of the samples was carried out using metaMS [18], an open-source pipeline for GC-MS based untargeted metabolomics. The results for each identified volatile compound were normalized based on the recovery of the 4-methyl-2-pentanol IS for each sample and expressed as a percent of the total volatiles recovered for each sample. Results in all cases were the averages of triplicate analysis.

2.5. Data Analysis

Each extraction technique, with or without salting out, were compared using non-polar and polar GC columns in relation to their ability to extract VOCs in these WMP samples. The results were expressed after normalization in relation to the IS. The sensitivity, selectivity, and reproducibility were compared in terms of: (i) the number of VOCs extracted by each technique, (ii) the percentage of each chemical class extracted by each technique, (iii) the specific identity of each VOC extracted by each technique, (iv) the total abundance of VOCs extracted by each technique (the overall abundance was calculated as the sum of the average abundance of every VOC peak area extracted by that technique, and expressed as a percentage. The extraction technique with the highest total abundance equated to 100% and the others were expressed as a percentage thereof), and (v) the average percentage relative standard deviation of each technique (taken from the relative standard deviation achieved for every VOC recovered in triplicate for each technique) as outlined in [19]. Principal component analysis (PCA) biplots of the volatile data were carried out to aid the visual association of volatile compounds using the "factoextra" and "FactoMineR" packages within R (v 3.4.1, R Foundation for Statistical Computing, Vienna, Austria). To visualize the selectivity of each technique in relation to the number of VOCs recovered, with or without salting out using the non-polar and polar GC columns, Venn diagrams were created with the 4 oval flower model using the Excel template (Microsoft Office, Redmond, WA, USA). Histograms outlining the percent of chemical classes of each extraction technique with or without salting out using the non-polar and polar GC columns were also created using Excel (Microsoft Office, Redmond, WA, USA).

3. Results and Discussion

3.1. Comparison of Volatile Compounds Extracted from Whole Milk Powder by Each Technique

A summary of all the VOCs identified by each technique, with and without salting out, in terms of percent of abundance, including standard deviations for each VOC, are provided in Table 2a (results using a non-polar GC column) and Table 2b (results using a polar GC column). In total, the number of individual VOCs identified in these samples across all four extraction techniques, with and without salting out and with both GC column polarities, was 85 (Table 3). This is considerably more VOCs than previously found in WMP, which, albeit, only used a single extraction technique [20,21]. Twenty-five VOCs were identified using salting out with SBSE (PDMS) using a non-polar GC column [21] and ten VOCs by HS-SPME (DVB/CAR/PDMS) with salting out using a non-polar GC column [20]. The 85 VOCs identified in this study consisted of 20 ketones, 18 aldehydes, 11 lactones, 11 alcohols, 7 esters, 6 benzene/phenols, 5 furans, 4 terpenes, 2 sulphur compounds, and 1 acid. Most VOCs were identified using the non-polar GC column (80) as opposed to the polar GC column (54) across all extraction techniques, independent of salting out (Table 3). A previous study comparing four volatile extraction techniques on natto (a fermented food) also found considerably more volatiles using a non-polar GC column than a polar GC column; 70 compared to 47 VOCs, with 40 VOCs recovered by both column polarities [22]. In this study, 30 (ethanol, 1-butanol, 1-octanol, (Z)-4-heptenal, (E)-2-octenal, (E)-2-nonenal, (E)-2-decenal and undecanal, benzeneacetaldehyde, benzyl alcohol, diacetyl, 2-hexanone, 2-octanone, 3-octanone, 2-tridecanone, 2-pentadecanone, 2-heptadecanone, γ-crotonolactone, δ-caprolactone, δ-nonalactone, γ-dodecalactone, δ-undecalactone, δ-tridecalactone, z-dairylactone, longifolene, methyl hexanoate, methyl pyruvate, 2-methyl furan, 2-pentyl furan, and acetic acid) VOCs were extracted, independent of salting out, using the non-polar GC column in comparison to the polar GC column across all four extraction techniques (Table 2a,b). In contrast, only six (1–3-pentanol, 1-nonanol, 2,3-pentanedione, δ-caprolactone, butyl acetate, and 2-ethyl furan) VOCs were extracted across all four extraction techniques, independent of salting out, on the polar GC column, but not on the non-polar GC column (Table 2a,b). Therefore, the VOCs were present in the extract(s) in each case, but did not interact with the particular GC column phase in order to be identified. This further highlights the significance of GC column polarity in volatile extraction/identification by GC-MS.

More VOCs were extracted and identified across all four techniques with salting out (75) and without salting out (72) with the non-polar GC column, than with salting out (48) and without salting (45) on the polar GC column (Table 4). Therefore, the impact of salting out was much less than the impact of column polarity in relation to the number of VOCs extracted. In general, salting out modifies the ionic strength of the sample solution with the aim of improving the extraction of polar VOCs, but may adversely impact the extraction of non-polar VOCs [23]. However, in practice the impact of salting out in relation to polar and non-polar VOCs is often unclear as many additional factors relating to the composition of the sample and the parameters of the specific extraction technique may also influence the extraction [23].

Table 2. (**a**) Identification of volatile compounds by each extraction technique with and without salting out using the non-polar GC column. (**b**) Identification of volatile compounds by each extraction technique with and without salting out using the polar GC column.

(a)

			RI		Identification	DI-HISorb S		DI-HISorb NS		HS-HiSorb S		HS-HiSorb NS		TD S		TD NS		HS-SPME S		HS-SPME NS	
No	**Compound**	**CAS**	**ORI**	**REF**	**Methods**	**% Area**	**% Stdev**	**% Area**	**% Stdev**	**% Area**	**% Stdev**	**% Area**	**% Stdev**	**% Area**	**% Stdev**	**% Area**	**% Stdev**	**% Area**	**% Stdev**	**% Area**	**% Stdev**
						Alcohols															
1	Ethanol	64-17-5	436	426	MS, RI, STD	0.73	0.16	0.27	0.26	0.72	0.31	0.53	0.38	nd	nd	nd	nd	nd	nd	nd	nd
2	1-Butanol	71-36-3	655	675	MS, RI, STD	nd	nd	nd	nd	1.06	0.35	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	1-Pentanol	71-41-0	762	768	MS, RI, STD	0.47	0.22	0.11	0.04	1.3	0.24	1.44	1.03	3.01	0.63	2.07	0.84	2.33	0.43	1.68	0.7
4	1-Hexanol	111-27-3	865	868	MS, RI, STD	0.17	0.07	nd	nd	1.03	0.46	0.72	0.025	1.17	0.37	0.46	0.23	0.87	0.33	0.77	0.3
5	2-Butoxy-ethanol	111-76-2	903	901	MS, RI	0.12	0.04	nd	nd	0.47	0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
6	1-Octen-3-ol	3391-86-4	977	981	MS, RI	0.07	0.04	nd	nd	nd	nd	0.39	0.31	nd	nd	nd	nd	nd	nd	nd	nd
7	2-Ethyl-1-hexanol	104-76-7	1026	1030	MS, RI, STD	0.31	0.14	0.25	0.07	1.96	0.37	nd	nd	8.61	1.67	7.2	1.37	nd	nd	nd	nd
8	1-Octanol	111-87-5	1067	1071	MS, RI, STD	0.22	0.15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
9	α-Terpineol	10482-56-1	1198	1192	MS, RI	nd	nd	nd	nd	nd	nd	0.57	0.28	0.32	0.07	0.26	0.04	nd	nd	nd	nd
						Aldehydes															
10	Acrolein	107-02-8	449	470	MS, RI	0.44	0.22	1.88	0.41	nd	nd	1.26	0.61	nd	nd	nd	nd	nd	nd	nd	nd
11	Butanal	123-72-8	578	596	MS, RI, STD	0.22	0.14	0.09	0.09	nd	nd	nd	nd	nd	nd	nd	nd	5	3.23	nd	nd
12	3-Methyl butanal	590-86-3	647	654	MS, RI, STD	nd	nd	nd	nd	0.61	0.21	0.56	0.19	1.31	0.41	1.33	0.56	4.58	1.41	5.35	2.65
13	Pentanal	110-62-3	697	697	MS, RI, STD	0.9	0.84	0.34	0.11	nd	nd	nd	nd	nd	nd	nd	nd	32.05	12.67	nd	nd
14	Hexanal	66-25-1	799	801	MS, RI, STD	0.94	0.48	0.65	0.26	7.42	4.64	7.45	5.21	6.64	1.66	6.95	2.62	11.43	8.06	16.66	9.82
15	4-Heptenal,(Z)-	6728-31-0	895	902	MS, RI	nd	nd	nd	nd	0.45	0.08	nd	nd	nd	nd	nd	nd	0.32	0.13	nd	nd
16	Heptanal	111-71-7	900	901	MS, RI, STD	0.69	0.15	0.59	0.08	7.11	1.68	7.73	2.42	8.48	1.38	9.38	2.32	5.87	1.93	9.89	2.19
17	Benzaldehyde	100-52-7	967	960	MS, RI, STD	0.15	0.04	0.2	0.05	1.3	0.6	1.86	0.4	1.13	0.37	1.81	1.18	0.34	0.14	1.26	0.12
18	Octanal	124-13-0	1002	1004	MS, RI, STD	0.31	0.08	0.29	0.06	3.21	0.94	3.08	1.09	3.49	0.99	3.78	2.13	0.71	0.12	1.77	0.46
19	Benzeneacetaldehyde	122-78-1	1048	1048	MS, RI, STD	nd	nd	0.04	0.01	nd	nd	1.43	0.64	nd	nd	nd	nd	nd	nd	nd	nd
20	2-Octenal,(E)-	2548-87-0	1059	1057	MS, RI	0.07	0.01	0.1	0.04	nd	nd	nd	nd	0.22	0.09	0.3	0.2	nd	nd	nd	nd
21	Nonanal	124-19-6	1103	1106	MS, RI, STD	1.27	0.39	1.18	0.21	17.52	3.1	16.91	3.47	17.93	2.58	18.26	3.65	1.5	0.24	3.27	0.32
22	2-Nonenal,(E)-	18829-56-6	1160	1160	MS, RI	0.17	0.11	0.11	0.04	0.41	0.13	nd	nd	0.22	0.15	0.2	0.06	nd	nd	nd	nd
23	Decanal	112-31-2	1204	1205	MS, RI, STD	0.36	0.1	0.28	0.1	5.27	1.96	4.57	1.4	1.24	0.46	1.76	0.79	nd	nd	nd	nd
24	2-Decenal,(E)-	3913-81-3	1262	1266	MS, RI	0.12	0.09	0.05	0.01	1.32	0.29	nd	nd	0.11	0.09	0.22	0.21	nd	nd	nd	nd
25	Undecanal	112-44-7	1306	1309	MS, RI, STD	0.07	0.02	0.05	0.02	0.49	0.06	0.48	0.11	0.12	0.03	0.21	0.06	nd	nd	nd	nd
26	2-Undecenal	2463-77-6	1364	1350	MS, RI, STD	0.2	0.09	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
27	Dodecanal	112-54-9	1407	1401	MS, RI, STD	0.12	0.03	0.11	0.03	0.41	0.03	0.53	0.1	0.25	0.07	0.3	0.17	nd	nd	nd	nd
						Benzene/Phenols															
28	Benzene	71-43-2	658	669	MS, RI, STD	0.36	0.17	0.31	0.2	nd	nd	1.33	0.47	6.77	3.33	8.08	5.53	nd	nd	6.2	2.7
29	Toluene	108-88-3	766	763	MS, RI, STD	0.1	0.02	0.09	0.05	0.51	0.24	0.6	0.12	4.54	0.73	3.64	1.08	1.72	0.67	1.6	1.07
30	p-Xylene	106-42-3	870	867	MS, RI, STD	nd	nd	nd	nd	nd	nd	0.77	0.78	3.39	0.6	3.14	0.8	nd	nd	nd	nd
31	o-xylene	95-47-6	898	900	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	1.74	0.75	1.23	0.34	nd	nd	nd	nd
32	Benzyl alcohol	108-95-2	974	995	MS, RI, STD	nd	nd	nd	nd	0.61	0.13	nd	nd	nd	nd	0.23	0.09	nd	nd	nd	nd
33	Phenol	100-51-6	1035	1037	MS, RI, STD	0.16	0.1	0.12	0.04	0.79	0.23	0.76	0.17	0.73	0.31	0.66	0.12	nd	nd	nd	nd
						Ketones															
34	Acetone	67-64-1	451	496	MS, RI, STD	1.62	0.61	nd	nd	8.11	2.62	6.5	1.02	nd	nd	nd	nd	5.4	0.65	nd	nd
35	Diacetyl	431-03-8	548	596	MS, RI, STD	nd	nd	nd	nd	nd	nd	1.75	0.57	3.72	1.67	3.1	0.68	nd	nd	nd	nd
36	Hydroxyacetone	116-09-6	663	657	MS, RI	0.68	0.31	1.03	0.71	0.12	0.11	2.91	2.49	nd	nd	nd	nd	nd	nd	nd	nd
37	2-Pentanone	107-87-9	684	687	MS, RI, STD	nd	nd	0.26	0.08	2.19	0.3	5.38	3.13	4.1	0.53	3.46	0.59	1.21	1.16	5.52	0.91

Table 2. *Cont.*

38	2-Butanone	108-10-1	733	740	MS, RI, STD	nd	nd	nd	nd	1.82	0.39	1.25	0.53	10.04	1.65	7.3	0.83	nd	nd	4.41	1.19
39	Methyl Isobutyl Ketone	108-10-1	735	740	MS, RI, STD	nd	nd	0.09	0.02	0.58	0.11	0.5	0.23	1.48	0.8	0.93	0.23	0.67	0.13	0.76	0.46
40	2-Hexanone	591-78-6	789	790	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.67	0.04	nd	nd
41	2-Heptanone	110-43-0	887	891	MS, RI, STD	1.23	0.28	1.14	0.27	11.95	0.97	10.34	3.18	2.62	0.41	3.85	0.6	13.12	2.71	16.17	6.16
42	2,3-Octanedione	585-25-1	981	967	MS, RI, STD	0.17	0.05	0.16	0.04	1.56	0.37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
43	3-Octanone	106-68-3	982	989	MS, RI	0.05	0.02	0.04	0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
44	2-Octanone	111-13-7	988	992	MS, RI, STD	nd	nd	0.07	0.02	0.39	0.16	0.45	0.07	0.56	0.13	0.55	0.28	nd	nd	nd	nd
45	3,5-Octadien-2-one,(E,E)-	30086-02-3	1069	1072	MS, RI	0.46	0.06	0.37	0.06	1.44	0.38	1.33	0.22	nd	nd	nd	nd	nd	nd	nd	nd
46	Acetophenone	98-86-2	1070	1079	MS, RI, STD	0.09	0.02	nd	nd	0.65	0.13	0.52	0.07	0.68	0.25	nd	nd	nd	nd	nd	nd
47	3,5-Octadien-2-one	38284-27-4	1076	1072	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.69	0.48	0.92	0.23	0.45	0.13
48	2-Nonanone	821-55-6	1088	1094	MS, RI, STD	0.3	0.09	0.51	0.12	3.19	0.19	3.43	0.18	0.54	0.12	0.69	0.05	0.93	0.1	2.33	0.21
49	2-Undecanone	112-12-9	1295	1294	MS, RI, STD	nd	nd	0.08	0.02	nd	nd	0.77	0.08	nd	nd	0.15	0.02	1.64	0.26	nd	nd
50	2-Tridecanone	593-08-8	1494	1480	MS, RI, STD	nd	nd	0.27	0.14	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
51	2-Pentadecanone	2345-28-0	1695	1689	MS, RI, STD	0.41	0.4	0.4	0.26	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
52	2-Heptadecanone	2922-51-2	1897	1878	MS, RI, STD	nd	nd	0.54	0.09	0.98	0.39	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
											Lactones										
53	γ-Crotonolactone	497-23-4	912	916	MS, RI	0.21	0.11	0.19	0.11	0.89	0.3	0.92	0.21	nd	nd	nd	nd	nd	nd	nd	nd
54	δ-Caprolactone	823-22-3	1097	1084	MS, RI	nd	nd	0.1	0.03	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
55	δ-Octalactone	698-76-0	1288	1288	MS, RI	nd	nd	0.63	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
56	δ-Nonalactone	3301-94-8	1394	1404	MS, RI	0.22	0.1	0.14	0.03	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
57	δ-Decalactone	705-86-2	1502	1506	MS, RI	31.87	2.9	28.34	4.87	3.81	0.5	3.06	0.45	nd	nd	nd	nd	0.29	0.05	1.05	0.22
58	δ-undecalactone	710-04-3	1602	1627	MS, RI	0.08	0.02	1.68	0.63	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
59	(Z) Dairy lactone	18679-18-0	1664	1675	MS, RI	0.64	0.23	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
60	γ-Dodecalactone	2305-(05)-7	1685	1674	MS, RI	8.56	10.18	13.9	15.58	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
61	δ-Dodecalactone	713-95-1	1717	1719	MS, RI	29.02	3.99	40.05	7.86	1.52	0.25	1.37	0.21	nd	nd	nd	nd	nd	nd	nd	nd
62	δ-Tridecalactone	7370-92-5	1824	1778	MS, RI	0.55	0.18	1.21	0.37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
63	δ-Tetradecalactone	2721-22-4	1930	1938	MS, RI	13.46	1.16	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
											Sulfurous Compounds										
64	Dimethyl sulfide	75-18-3	519	510	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.68	0.65	3.93	1.68
65	Dimethyl disulfide	624-92-0	743	739	MS, RI, STD	0.09	0.02	0.1	0.02	0.19	0.09	nd	nd	1.74	0.32	4.62	1	1.7	0.86	0.85	0.15
											Terpenes										
66	α-Pinene	80-56-8	939	930	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.58	0.47	1.93	1.24
67	3-Carene	13466-78-9	1015	1009	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.49	0.17	1.85	1.02
68	D-Limonene	5989-27-5	1032	1022	MS, RI, STD	0.01	0.02	0.05	0.01	0.33	0.06	0.28	0.06	2.52	2.55	1.18	0.89	0.27	0.04	0.3	0.13
69	Longifolene	475-20-7	1439	1432	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	0.14	0.03	0.17	0.08	nd	nd	nd	nd
											Acids										
70	Acetic acid	64-19-7	535	629	MS, RI, STD	0.21	0.05	0.23	0.14	1.17	1.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
											Esters										
71	Methyl butanoate	623-42-7	716	724	MS, RI, STD	nd	nd	nd	nd	nd	nd	1.05	0.3	0.22	0.04	1.16	0.4	1.5	0.55	2.44	1.14
72	Methyl pyruvate	108-10-1	735	740	MS, RI	0.29	0.19	0.16	0.08	0.54	0.36	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
73	Ethylbenzene	100-41-4	859	851	MS, RI, STD	nd	nd	nd	nd	0.53	0.11	nd	nd	0.22	0.15	0.68	0.41	nd	nd	nd	nd
74	Methy hexanoate	106-70-7	918	922	MS, RI	nd	nd	nd	nd	nd	nd	1.31	0.41	nd	nd	nd	nd	0.83	0.1	3.36	1.88
75	Methyl octanoate	111-11-5	1117	1126	MS, RI	nd	nd	nd	nd	nd	nd	0.53	0.43	nd	nd	nd	nd	nd	nd	nd	nd
76	Methyl hexadecanoate	112-39-0	1915	1909	MS, RI	nd	nd	nd	nd	2.16	0.77	2	0.32	nd	nd	nd	nd	nd	nd	nd	nd
											Furans										
77	2-Methyl-furan	534-22-5	602	604	MS, RI, STD	nd	nd	0.17	0.04	0.52	0.42	nd	nd	nd	nd	nd	nd	nd	nd	7.59	4.83
78	2-Pentyl-furan	3777-69-3	989	991	MS, RI, STD	nd	nd	0.05	0.01	0.43	0.19	0.44	0.08	nd	nd	nd	nd	nd	nd	nd	nd
79	2-Furanmethanol	98-0-0	851	850	MS, RI, STD	0.65	0.59	0.59	0.75	0.49	0.35	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
80	Furfural	98-01-1	833	852	MS, RI, STD	0.39	0.19	0.36	0.35	0.51	0.11	0.93	0.24	nd	nd	nd	nd	nd	nd	nd	nd

Table 2. *Cont.*

(b)																				
Alcohols																				
1	α-Terpineol	10482-56-1	1206	1192	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	0.73	0.29	nd	nd	nd	nd	nd	nd
2	1-Pentanol	71-41-0	1262	1250	MS, RI, STD	nd	nd	nd	nd	2.34	1.86	2.17	3.35	nd	nd	nd	nd	nd	nd	nd	nd
3	1-Hexanol	111-27-3	1365	1355	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	1.11	0.4	0.88	0.38	1.44	0.86	0.87	0.91
4	1-Penten-3-ol	111-27-3	1365	1355	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	1.89	1.02	1.25	0.95	2.06	1.76	nd	nd
5	2-Butoxy-ethanol	111-76-2	1423	1405	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.45	1.4	nd	nd	nd	nd
6	1-Octen-3-ol	3391-86-4	1460	1450	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.67	0.1	nd	nd	nd	nd
7	2-Ethyl-1-hexanol	71-41-0	1502	1491	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	12.02	1.89	15.69	3.3	4.18	0.51	2.18	0.81
8	1-Nonanol	143-08-8	1673	1660	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.81	0.33	nd	nd	nd	nd
9	Acrolein	107-02-8	449	470	MS, RI	0.2	0.24	0.31	0.05	4.55	2.02	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	Butanal	123-72-8	578	596	MS, RI, STD	nd	nd	0.12	0.03	nd	nd	nd	nd	1.04	0.08	1.3	0.24	nd	nd	nd	nd
11	3-Methyl-butanal	590-86-3	652	654	MS, RI, STD	0.28	0.05	nd	nd	nd	nd	nd	nd	0.59	0.48	1.08	0.65	2.2	1.13	17.4	17.85
12	Pentanal	110-62-3	994	979	MS, RI, STD	0.68	0.26	0.52	0.23	1.68	1.05	2.22	1.93	7.38	2.74	nd	nd	11.23	5.97	nd	nd
13	Hexanal	66-25-1	1098	1083	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	2.6	0.64	3.87	0.58	5.9	0.33	4.55	6.02
14	Heptanal	111-71-7	1204	1184	MS, RI, STD	0.57	0.1	0.4	0.13	9.62	4.17	13.91	6.8	4.01	0.92	4.81	0.89	10.47	3.64	24.2	10.48
15	Octanal	124-13-0	1310	1289	MS, RI, STD	nd	nd	0.4	0.06	2.34	0.43	4.32	0.98	1.85	0.78	2.43	1.09	nd	nd	nd	nd
16	Nonanal	124-19-6	1416	1391	MS, RI, STD	1.02	0.24	0.89	0.31	1.27	0.96	8.41	6.43	10.12	3.05	12.09	2.97	0.62	0.37	nd	nd
17	Decanal	112-31-2	1523	1498	MS, RI, STD	0.48	0.12	0.61	0.31	16.88	9.22	8.54	12.98	1.44	0.24	2.99	1.03	nd	nd	nd	nd
18	Benzaldehyde	100-52-7	1570	1520	MS, RI, STD	0.52	0.1	0.33	0.07	5.63	4.25	4.56	4.43	1.21	0.31	2.55	0.45	0.9	0.11	2.28	1.06
19	Dodecanal	112-54-9	1733	1710	MS, RI, STD	nd	nd	nd	nd	0.31	0.47	6.08	1.32	nd	nd	nd	nd	nd	nd	nd	nd
Benzene/Phenols																					
20	Toluene	108-88-3	766	763	MS, RI, STD	1.1	0.17	nd	nd	nd	nd	nd	nd	0.56	0.16	0.75	0.2	0.53	0.41	2.62	0.96
21	Benzene	71-43-2	955	957	MS, RI, STD	0.34	0.2	0.26	0.17	nd	nd	nd	nd	1.49	0.37	2	0.72	nd	nd	nd	nd
22	p-Xylene	106-42-3	1155	1138	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.86	0.17	nd	nd	nd	nd
23	o-Xylene	95-47-6	1156	1186	MS, RI, STD	0.35	0.04	nd	nd	nd	nd	nd	nd	2.02	1.21	nd	nd	nd	nd	nd	nd
24	Phenol	108-95-2	2042	2039	MS, RI, STD	nd	nd	nd	nd	1.14	1.37	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ketones																					
25	Hydroxyacetone	116-09-6	663	657	MS, RI	2.68	1.68	2.49	1.14	nd	nd	0.51	0.79	nd	nd	nd	nd	nd	nd	nd	nd
26	2-Pentanone	107-87-9	684	687	MS, RI, STD	0.74	0.43	nd	nd	nd	nd	nd	nd	5.18	3.58	nd	nd	nd	nd	8.65	2.87
27	Methyl isopropyl ketone	108-10-1	735	740	MS, RI, STD	nd	nd	0.45	0.18	nd	nd	nd	nd	nd	nd	nd	nd	8.97	1.96	nd	nd
28	Acetone	67-64-1	825	819	MS, RI, STD	1.6	0.62	1.17	0.33	2.89	1.09	3.46	1.67	26.04	7.92	29.76	8.27	9.61	2.67	6.07	5.05
29	2-Heptanone	110-43-0	887	891	MS, RI, STD	3.04	1.15	0.36	0.1	1.44	0.51	1.84	0.79	6.33	1.89	nd	nd	17.94	4.91	nd	nd
30	2-Butanone	108-10-1	913	907	MS, RI, STD	0.28	0.17	nd	nd	nd	nd	nd	nd	6.88	2.11	8.38	2.44	5.97	4.05	5.39	1.2
31	Acetophenone	98-86-2	1070	1079	MS, RI, STD	0.43	0.07	nd	nd	nd	nd	nd	nd	0.7	0.22	nd	nd	nd	nd	nd	nd
32	2,3-Pentanedione	600-14-6	1073	1058	MS, RI	nd	nd	nd	nd	nd	nd	nd	nd	0.82	0.34	0.95	0.33	nd	nd	nd	nd
33	2-Nonanone	821-55-6	1410	1390	MS, RI, STD	1.13	0.56	0.98	0.23	nd	nd	nd	nd	0.95	0.11	1.8	0.26	2.53	0.62	3.63	1.59
34	3,5-Octadien-2-one	38284-27-4	1549	1522	MS, RI	nd	nd	nd	nd	11.26	11.15	7.02	5.4	0.95	0.67	0.83	0.28	nd	nd	2.29	0.94
35	3,5-Octadien-2-one,(E,E)-	30086-02-3	1551	1570	MS, RI	1.38	0.83	1	0.47	2.84	2.36	0.66	1.32	nd	nd	nd	nd	0.92	0.74	nd	nd
36	2-Undecanone	112-12-9	1622	1598	MS, RI, STD	nd	nd	0.68	0.17	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Lactones																					
37	δ-Caprolactone	823-22-3	1864	1791	MS, RI	0.25	0.24	0.33	0.09	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
38	δ-Octalactone	698-76-0	2037	1976	MS, RI	nd	nd	nd	nd	1.53	2.29	nd	nd	nd	nd	nd	nd	4.21	0.26	nd	nd
39	δ-Decalactone	705-86-2	2242	2190	MS, RI	nd	nd	nd	nd	12.46	3.67	14.39	3.58	nd	nd	nd	nd	nd	nd	nd	nd
40	δ-Dodecalactone	713-95-1	2570	2436	MS, RI	62.94	1.99	62.33	1.64	5.56	4.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
41	δ-Tetradecalactone	7370-92-5	2892	2688	MS, RI	19.61	1.57	24.59	2.66	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 2. *Cont.*

											Sulfurous Compounds									
42	Dimethyl disulfide	624-92-0	743	739	MS, RI, STD	0.38	0.04	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
43	Dimethyl sulfide	75-18-3	755	754	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	0.36	0.45	0.69	0.34	4.24	2	3.72	0.91
											Terpenes									
44	α-Pinene	80-56-8	1035	1028	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.9	0.22	2.09	1.66
45	3-Carene	13466-78-9	1152	1147	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.64	0.19	1.45	1.94
46	D-Limonene	5989-27-5	1218	1200	MS, RI, STD	nd	nd	0.16	0.07	nd	nd	nd	nd	0.8	0.41	1.1	0.27	nd	nd	7.72	4.94
											Esters									
47	Methyl butanoate	623-42-7	1001	982	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.46	0.87	4.9	2.72
48	Butyl acetate	123-86-4	1087	1074	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	0.93	0.43	nd	nd	nd	nd	nd	nd
49	Ethylbenzene	100-41-4	1120	1129	MS, RI, STD	0.06	0.01	nd	nd	nd	nd	nd	nd	0.66	0.06	0.8	0.11	nd	nd	nd	nd
50	Methyl hexadecanoate	112-39-0	1915	1909	MS, RI	nd	nd	nd	nd	3.67	1.28	1.13	1.92	nd	nd	nd	nd	nd	nd	nd	nd
51	Methyl octanoate	111-11-5	1407	1385	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.05	0.07	nd	nd
											Furans									
52	2-Ethyl-furan	3208-16-0	968	950	MS, RI, STD	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.01	0.41	nd	nd
53	Furfural	98-01-1	1497	1461	MS, RI, STD	nd	nd	0.8	0.36	8.91	7.71	17.33	10.71	nd	nd	nd	nd	nd	nd	nd	nd
54	2-Furanmethanol	98-00-0	1681	1660	MS, RI, STD	nd	nd	0.81	0.73	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Results are expressed as relative abundance normalized to internal standard (% area, % relative standard deviation). CAS (Chemical Abstracts Service number). MS (identity confirmed by mass spectra to an in-house library). RI (linear retention index as determined). ORI (linear retention index as determined in this study). REF (relevant linear retention index as published reference, if available). STD (an internal standard was used to confirm identification). nd (not determined).

Table 3. The numbers of volatile organic compounds extracted in whole milk powder samples.

No of VOCs Extracted	Non-Polar GC Column	Polar GC Column
With salting out	75	48
Without salting out	72	45
Total	80	54
Overall Total	85	

Table 4. Numbers, abundance, and reproducibility of volatile organic compounds in whole milk powder samples extracted by each technique with and without salting out and for polar and non-polar GC columns.

NON-POLAR GC COLUMN								
Extraction Technique	**HS-SPME S**	**TD S**	**Di-HiSorb S**	**HS-HiSorb S**	**HS-SPME NS**	**TD NS**	**Di-HiSorb NS**	**HS-HiSorb NS**
No of VOCs	28	34	49	46	25	36	51	42
Abundance %	1.7	1.3	34.2	7.5	3.9	2.2	100	11.6
Average RSD %	39.3	33.7	45.3	33.5	38.3	38.1	40.5	35.2
POLAR GC COLUMN								
Extraction Technique	**HS-SPME S**	**TD S**	**Di-HiSorb S**	**HS-HiSorb S**	**HS-SPME NS**	**TD NS**	**Di-HiSorb NS**	**HS-HiSorb NS**
No of VOCs	23	28	23	19	17	25	22	16
Abundance %	1.2	2.5	39.7	4	1.3	3.1	100	7.7
Average RSD %	37.4	39.1	37.6	71.1	63.8	32.5	34.6	90

No. of VOCs (number of volatile organic compounds). Abundance %, (the greatest abundance achieved by a single extraction technique equated to 100% and the remaining extraction techniques were expressed as a percentage thereof). Average RSD % (the average percentage relative standard deviation of all VOCs for extraction technique).

3.2. The Percentage of Each Chemical Class Extracted from Whole Milk Powder by Each Technique

Figure 1a is histogram highlighting the breakdown of the percentage of each chemical class extracted by each of the four techniques using the non-polar GC column with and without salting out. Figure 1b is the corresponding figure for the polar GC column. It is immediately apparent that significant differences existed in relation to the type and percentage of each chemical class extracted by each technique, influenced by GC column polarity and, to a lesser extent, salting out. All DI-HiSorb techniques (DI-HiSorb S and DI-HiSorb NS), independent of GC column polarity, were characterized by the large volume of lactones extracted (>82%), which differs considerably to all of the other extraction techniques. A similar result was found for SBSE, which is a comparable technique to DI-HiSorb that also used PDMS as the sorbent phase [19]. The only other significant number of chemical classes extracted by DI-HiSorb NS were aldehydes, ketones, and furans, but DI-HiSorb NS did not extract any alcohols, sulphur compounds, acids, terpenes, or esters with the non-polar GC column (but did extract low levels, from 0.5–0.63%, with the polar GC column). The DI-HiSorb S slightly modified the percentage recovery of some chemical classes in comparison to the DI-HiSorb NS. The percentages of chemical classes extracted by DI-HiSorb (DI-HiSorb S and DI-HiSorb NS) using the non-polar GC column were similar independent of salting out (although slightly more alcohols were extracted with salting out). The percentage of each chemical class extracted by HS-HiSorb (HS-HiSorb S and HS-HiSorb NS) for each GC column polarity differed considerably to that attained by DI-HiSorb. This same trend was also apparent when comparing HSSE (similar to HS-HiSorb) and SBSE (similar to DI-HiSorb) [19]. HS-HiSorb S and HS-HiSorb NS attained a much lower percentage of lactones on the polar GC column (~14–20%) and with the non-polar GC column (~5–6%) than DI-HiSorb S and DI-HiSorb NS. HS-HiSorb S and HS-HiSorb NS had a much higher percentage recovery of aldehydes (~42–48%) than DI-HiSorb S and DI-HiSorb NS across both GC column polarities. The percentage of ketones extracted by HS-HiSorb S and HS-HiSorb NS varied considerably depending upon GC column polarity with levels at ~32–35% with the non-polar GC column, and ~14–19% with the polar GC column. The greatest difference in the percentage recovery of chemical classes between HS-HiSorb S and HS-HiSorb NS in relation to GC column polarity, apart from lactones, was for the recovery of furans, with only 1–2% recovered using the non-polar GC column, but

9–17% recovered on the polar GC column. The percentage recovery of esters by HS-HiSorb S and HS-HiSorb NS were relatively high in comparison to the other extraction methods at ~3–5% for the non-polar GC column and ~2–4% for the polar GC column. HS-HiSorb NS did not recover any terpenes, sulphur compounds, acids, or benzene/phenol compounds using the polar GC column, nor sulphur compounds or acids using the non-polar GC column. TD S and TD NS were characterized as having a high percentage recovery of aldehydes (~30–45%), ketones (~21–48%), and alcohols (~10–22%) that varied with both GC column polarity and salting out. TD, independent of salting out, recovered the highest percentage of benzene/phenol compounds using the non-polar GC column in comparison to all the other extraction techniques. TD S or TD NS did not extract any lactones, acids, or furans independent of salting out or GC column polarity. The impact of salting out was minimal in relation to TD, however the percentage of alcohols decreased with salting out using the polar GC column, but increased using the non-polar GC column. Overall, the combination of TEN/CAR should enable a wide range of VOCs to be recovered, as TEN is particularly suited to the extraction of non-polar and slightly polar VOCs, apart from very low molecular weight (<C6) VOCs, which CAR can extract [24]. The percentage recovery of many chemical classes differed most in relation to both GC column polarity and salting out for HS-SPME than any of the other extraction techniques. Overall, HS-SPME recovered all chemical classes except for acids, independent of salting out and GC column polarity, or furans and lactones by HS-SPME NS using the polar GC column, or furans by HS-SPME S using the non-polar GC column. Overall HS-SPME was characterized by a high percentage recovery of aldehydes (~31–62%), which was reduced with the inclusion of salting out independent of both GC column polarities. For HS-SPME S, the percentage recovery of ketones (25–46%) was much higher using the polar GC column. HS-SPME NS also recovered many more terpenes independent of GC column polarity. Previous studies noted that the DVB/CAR/PDMS multiphase SPME fibers tend to extract the most volatile low boiling point VOCs more effectively [25], which corresponds with the results of this study.

No acids were recovered by any technique using the polar GC-column, however it worth pointing out that only one acid (acetic acid) was identified in these WMP samples.

3.3. The Relationship between the Individual Volatile Compound Chemical Classes Extracted by Each Technique in the Whole Milk Powder

Figure 2a,b are principal component analysis (PCA) plots, highlighting the associations of the different extraction techniques with each chemical class. The chemical class data used to generate the PCA was based on the percentage of each chemical class, rather than individual VOCs determined for each technique (with and without salting out for each GC column polarity), to visualize the associations between chemical class and individual extraction techniques, rather than individual VOC. Figure 2a relates to each chemical class with and without salting out using the non-polar GC column. The total level of discrimination was 44.8% (PCA 1 23.6% and PCA 2 20.6%). It is immediately apparent that salting out did not have a major impact on the individual extraction techniques, as with and without salting out (S and NS) for each technique are very closely associated with each other. Both DI-HiSorb S and DI-HiSorb NS were most strongly associated with lactones as previously mentioned, and were separate from HS-HiSorb S and HS-HiSorb NS, which were not strongly associated with any chemical class, but more so with esters and ketones. Although both HS-HiSorb techniques appeared closely associated with the chemical group acids, this was more a reflection of using the overall percentage of each chemical class data, the fact that only one VOC (acetic acid) made up this chemical class, and due to the relationship of this acid with the other extraction techniques. In fact, HS-HiSorb NS did not extract acetic acid. TD (TD S and TD NS) were most strongly associated with aldehydes, benzene/phenols, alcohols, and ketones, while HS-SPME (HS-SPME S and HS-SPME NS) were most closely associated with terpenes and sulphur compounds. Although the HS-SPME techniques did extract a high percentage of ketones and aldehydes, the association was less obvious as the relationship of these chemical classes to the other extraction techniques

also influenced their position on the PCA (as stated earlier, the overall percentage of each chemical class data was used to generate the PCA rather than individual VOC).

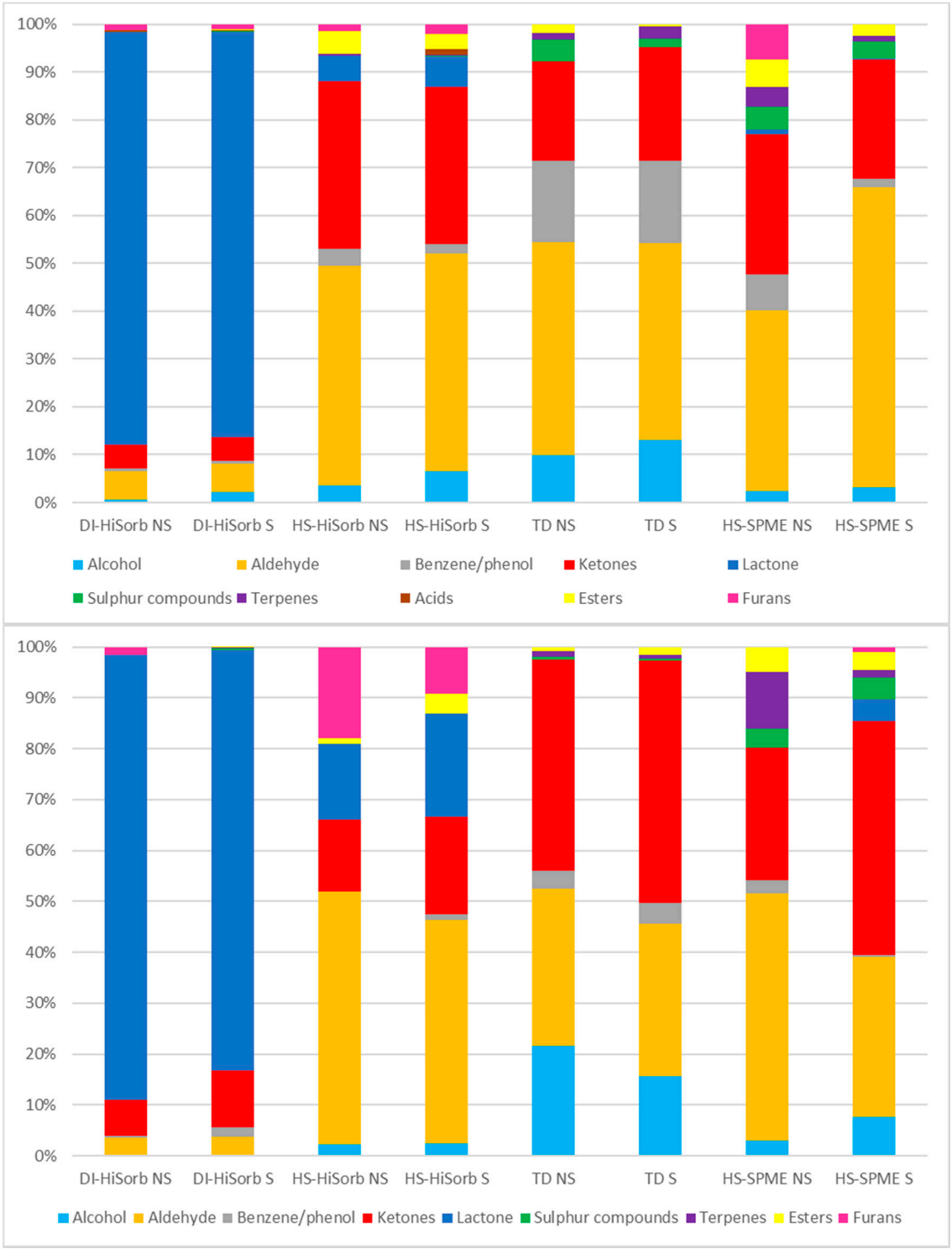

Figure 1. (**a**) The percentage of each chemical class extracted by the four extraction techniques using the non-polar GC column with and without salting out, and (**b**) the percentage of each chemical class extracted by the four extraction techniques using the polar GC column with and without salting out. Direct Immersion HiSorb without salting out (DI-HiSorb NS), Direct Immersion HiSorb with salting out (DI-HiSorb S), Headspace HiSorb without salting out (HS-HiSorb NS), Headspace HiSorb with salting out (HS-HiSorb S), Thermal Desorption without salting out (TD-NS), Thermal Desorption with salting out (TD S), Headspace Solid Phase Microextraction without salting out (HS-SPME NS), Headspace Solid Phase Microextraction with salting out (HS-SPME S).

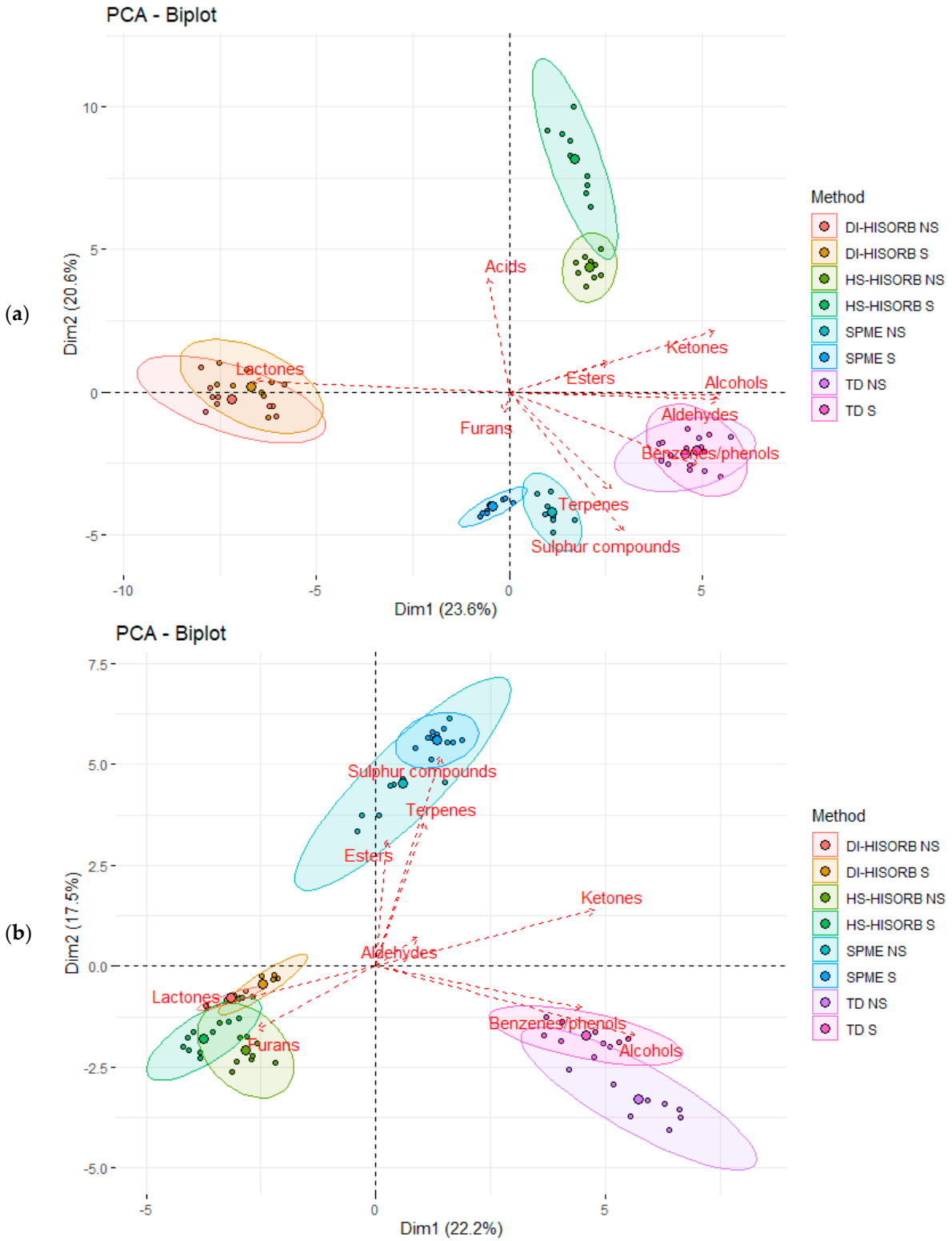

Figure 2. (**a**) Principal component analysis of the volatile organic compounds (as per chemical class) per individual extraction technique, with and without salting out, using the non-polar GC column, and (**b**) principal component analysis of the volatile organic compounds (as per chemical class) per individual extraction technique, with and without salting out, using the polar GC column. Direct Immersion HiSorb without salting out (DI-HiSorb NS), Direct Immersion HiSorb with salting out (DI-HiSorb S),Headspace HiSorb without salting out (HS-HiSorb NS), Headspace HiSorb with salting out (HS-HiSorb S), Thermal Desorption without salting out (TD-NS), Thermal Desorption with salting out (TD S), Headspace Solid Phase Microextraction without salting out (HS-SPME NS), Headspace Solid Phase Microextraction with salting out (HS-SPME S).

Figure 2b highlights the same associations of chemical classes with each extraction technique with and without salting out, but using the polar GC column. The level of overall discrimination was less, at 39.7% (PCA 1 22.2% and PCA 2 17.5%), than that achieved in Figure 2a. As acetic acid was found using the polar GC column, the acid chemical class is not present. Some similar patterns are evident with the polar GC column as found with the non-polar GC column. It is also evident that the impact of salting out was minimal due to the close association of each individual technique with and without salting out (S and NS). For the polar GC column, both DI-HiSorb (DI-HiSorb S and DI-HiSorb NS) and HS-HiSorb (HS-HiSorb S and HS-HiSorb NS) were much more closely associated with lactones and furans. However, there were some anomalies in that DI-HiSorb, independent of salting out, had little or no recovery of furans, and that both DI-HiSorb and HS-HiSorb recovered significant levels of ketones, which were not reflected in the PCA. As previously mentioned, this is due to the use of the percentage of chemical class data rather than individual VOC data to create the PCA. HS-SPME (HS-SPME S and HS-SPME NS) were most closely associated with sulphur compounds, terpenes, and esters, but also with aldehydes and ketones. TD (TD S and TD NS) were most closely associated with alcohols and benzene/phenol VOCs, and, to a lesser extent, with aldehydes and ketones.

3.4. The Selectivity of Each Extraction Technique

Figure 3 is a series of Venn diagrams representing the selectivity of each technique in relation to (a) with salting out using the non-polar GC column, (b) without salting out using the non-polar GC column, (c) with salting out using the polar GC column, and (d) without salting out using the polar GC column. These figures highlight commonalities in relation to VOCs extracted by each technique and those recovered only by each individual extraction technique. It is immediately apparent that more VOCs were extracted using the non-polar GC columns (Figure 3a,b) than with the polar GC column (Figure 3c,d), as previously stated. However, these figures give a better insight into the discrepancies and commonalities with regard to the numbers of VOCs extracted across all four techniques. The greatest number of VOCs were associated with the two HiSorb techniques (DI-HiSorb and HS-HiSorb).

More commonalities were evident in relation to specific VOCs between both Hi-Sorb techniques, which is not surprising as they both utilize the same sorbent phase (PDMS), despite the fact that, in general, PDMS is regarded as less useful for the recovery of polar VOCs [26]. Thus overall, Hi-Sorb was quite effective for the general recovery of VOCs in WMP. Considerably fewer synergies were evident between both Hi-Sorb techniques and HS-SPME and TD. As mentioned, PDMS is thought to be more effective for the recovery of less polar VOCs than HS-SPME with multiple fiber phases [23]. HS-SPME appeared to be very effective at recovering terpenes and sulphur compounds independent of column polarity and salting out. While quite poor at recovering lactones, furan, and acids, this was also dependent upon GC column polarity and, to a much lesser extent, salting out. The DVB/CAR/PDMS multiphase SPME fibers tended to extract very volatile low boiling point VOCs more effectively [25]. In addition, as DVB is a polar porous coating, it is quite efficient in extracting polar compounds and, thus, was useful for sulphur VOCs [19,25,27], as evident in this study. The TEN components of the TD phase were less useful for very volatile VOCs, but compensated to some extent by the inclusion of CAR in the packing material [24].

In summary, 12 VOCs were extracted by every technique (1-pentanol, 1-hexanol, hexanal, heptanal, octanal, nonanal, benzaldehyde, toluene, 2-heptanone, 2-nonanone, dimethyl sulfide, and D-limonene) (Table 2a) with salting out using the non-polar GC column (Figure 3a). Thirteen 13 VOCs (1-pentanol, hexanal, heptanal, octanal, nonanal, benzaldehyde, toluene, benzene, 2-pentanone, 2-heptanone, methyl isopropyl ketone, 2-nonanone, and D-limonene) (Table 2a) were extracted by every technique without salting out using the non-polar GC column (Figure 3b). Whereas, in relation to the polar GC column, only seven VOCs (pentanal, heptanal, nonanal, benzaldehyde, decanal, acetone,

and 2-heptanone) (Table 2b) were extracted with salting out (Figure 3c), and only three VOCs (heptanal, benzaldehyde, and acetone) (Table 2b) without salting out (Figure 3d).

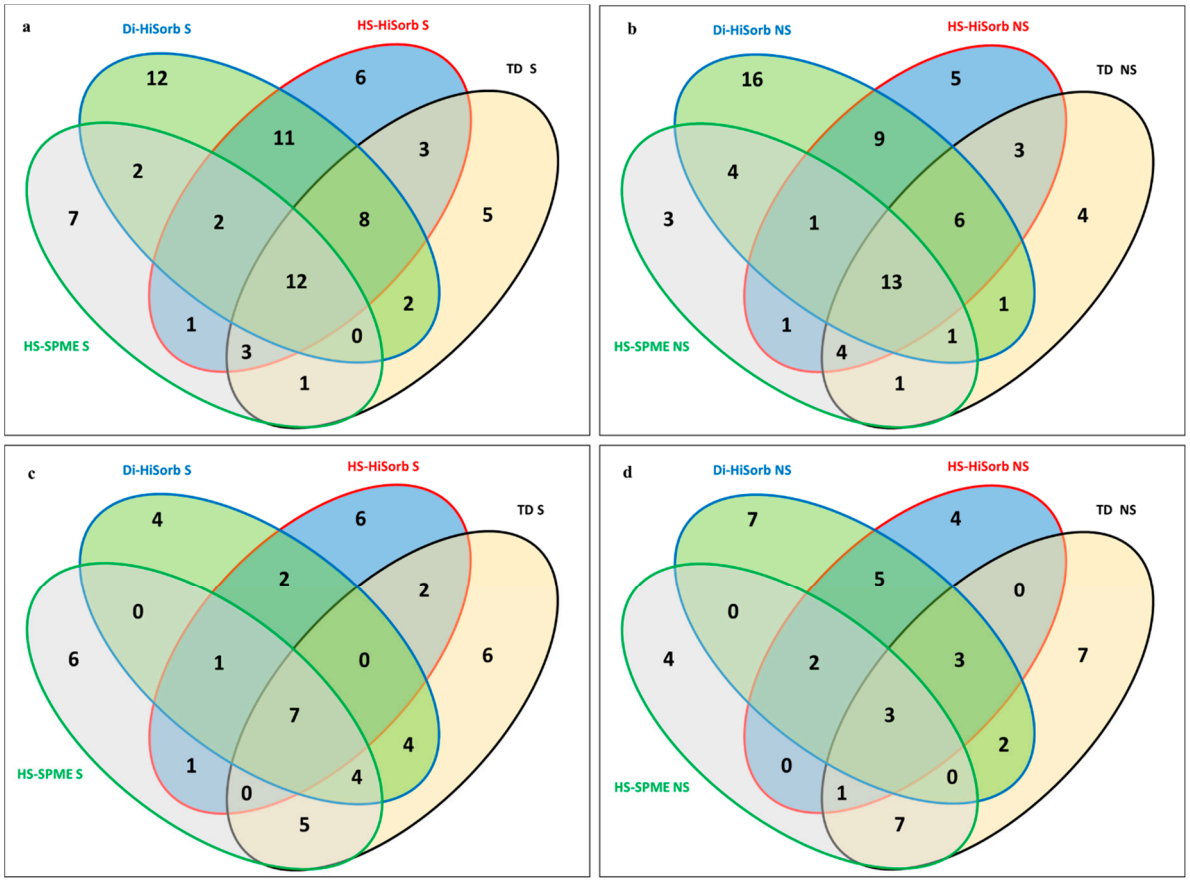

Figure 3. Venn diagrams of the number of volatile organic compounds extracted by each technique, including commonalities (**a**) with salting out using the non-polar GC column, (**b**) without salting out using the non-polar GC column, (**c**) with salting out using the polar GC column, and (**d**) without salting out using the polar GC column. Direct Immersion HiSorb without salting out (DI-HiSorb NS), Direct Immersion HiSorb with salting out (DI-HiSorb S), Headspace HiSorb without salting out (HS-HiSorb NS), Headspace HiSorb with salting out (HS-HiSorb S), Thermal Desorption without salting out (TD-NS), Thermal Desorption with salting out (TD S), Headspace Solid Phase Microextraction without salting out (HS-SPME NS), Headspace Solid Phase Microextraction with salting out (HS-SPME S).

The only VOCs extracted by all four extraction techniques independent of GC column polarity and salting out were heptanal and benzaldehyde (Table 2a,b). This likely reflects a combination of their relative abundance and chemical properties, which enabled them to be more easily recovered by each technique, despite the range of different phases and GC column polarities.

3.5. The Abundance of Volatile Organic Compounds in Whole Milk Powder by Each Extraction Technique

The greatest abundance of VOCs extracted across all techniques was achieved by DI-HiSorb NS, independent of GC column polarity (Table 4). As mentioned, all other abundances were expressed as a percentage of the technique with the greatest abundance (i.e., DI-HiSorb NS equated to 100% abundance). The abundance of VOCs recovered by DI-HiSorb NS was also impacted by GC column polarity, as abundances achieved by the non-polar GC column were ~41% lower than that achieved by the polar GC column (data not shown). Therefore, even though more VOCs were recovered using the non-polar GC column by DI-HiSorb NS, the total abundances were lower. The average total abundance for DI-HiSorb S, HS-HiSorb NS, HS-HiSorb S, HS-SPME NS, TD NS, HS-SPME S and

TD S for the non-polar GC column was 34.2%, 11.6%, 7.5%, 3.9%, 2.2%, 1.7%, and 1.3%, respectively. A similar trend was evident for the polar GC column, where DI-HiSorb S, HS-HiSorb NS, HS-HiSorb S, TD NS, TD S, HS-SPME NS, and HS-SPME S were 39.7%, 7.7%, 4.0%, 3.1%, 2.5%, 1.3%, and 1.2%, respectively, of that attained by DI-HiSorb NS (100%). The much greater abundance of the DI-HiSorb technique appears mainly due to the advantages of DI over HS, namely, the high capacity of the phase (which is much greater than SPME), and the selectivity of the phases' ability to extract lactones (more volume and quantities of lactones). The abundance of DI-HiSorb was impacted by salting out, as the addition of salt decreased abundances by approximately two thirds. The abundances of TD and HS-SPME were similar and slightly less than those achieved for HS-HiSorb. Therefore, the dynamic nature of TD, in comparison to the static HS-SPME and HS-HiSorb techniques, did not significantly impact VOC abundance. Differences in capacity and selectivity of the difference phases had a lesser impact than DI versus HS on abundance. A study comparing the SBSE (similar to DI-HiSorb), HSSE (similar to HS-HiSorb), and HS-SPME also found a similar trend for the extraction of fruit VOCs [28], in that SBSE extracted more and a greater abundance of VOCs than HSSE and HS-SPME.

3.6. The Reproducibility of Each Extraction Technique

The reproducibility of each technique was assessed by comparing the average percent relative standard deviation, (average of the percent relative standard deviation of every VOC for each technique) (Table 4) for each extraction technique in relation to column polarity and salting out. In terms of the non-polar GC column with and without salting out, the average standard deviation varied from 33.5% (HS-HiSorb S) to 45.3% (DI-HiSorb S). The average standard deviation range was greater for the polar GC column with or without salting out, from 32.5% for TD NS to 90.0% for HS-HiSorb NS. Overall reproducibility was lower for the HS techniques (HS-HiSorb S, HS-SPME NS, and HS-HiSorb NS) for the polar GC column than any of the other techniques. A recent study on spray dried sheep milk found that the average reproducibility (again, based on average relative standard deviation) of HS-SPME and SBSE was better than HSSE using a non-polar column [19].

It must be stated that the average percent relative standard deviation is a relatively crude approach to assess reproducibility. Nevertheless, it was used in this study for comparative convenience across the four techniques due to the number of factors assessed and the significant number of VOCs extracted. The average percentage relative standard deviation does not account for differences in the numbers, abundances, or the selectivity of each technique (impacted by the chemical properties of VOCs and the phases used in each extraction technique) all of which can have an impact on reproducibility. Thus, the individual relative percentage standard deviation values attained for each VOC across each technique with or without salting out for each GC column polarity provided a more in-depth, true reflection of reproducibility (Table 2a,b).

4. Conclusions

The evaluation of WMP by these four extraction techniques has highlighted the extent of VOCs present, which consisted mainly of ketones, aldehydes, lactones, and alcohols with lower numbers of esters, benzenes, phenols, furans, terpenes, sulphur compounds, and one acid. The overall difference in selectivity between the extraction techniques also highlights the need for multiple extraction techniques in order to obtain as true a representation of the complete volatile profile as possible. This is a simple fact, but often forgotten in volatile research of dairy and other foods. In relation to the four techniques, DI-HiSorb, HS-HiSorb, TD, and HS-SPME, the impact of GC column polarity was far greater than the impact of salting out under the conditions evaluated. It would appear that, unless specifically required to target a VOC (or specific VOCs) using a polar GC column, significantly more VOC information can be attained than using a non-polar GC column. As stated, the impact of salting out was minimal, but did vary depending upon the extraction technique, GC column polarity, and in relation to individual VOCs. Overall, the greatest number of VOCs

were extracted by DI-HiSorb using the non-polar GC column, and slightly more without salting out. However, even though the numbers of VOCs extracted by DI-HiSorb was considerably reduced using the polar GC column, the overall abundance of VOCs was higher than achieved with the non-polar GC column. A key element as to why the overall abundances and numbers of VOCs were generally higher with DI-HiSorb, as opposed to the other techniques, was the ability of DI-HiSorb to extract large quantities and volumes of lactones. Only TD failed to extract any lactones in these WMP samples. As HS-HiSorb has the same sorptive PDMS phase as DI-HiSorb, the different conditions between DI and HS was a key factor influencing the effectiveness of each of these techniques in extracting lactones and other VOCs. It appears that it was more difficult to extract some VOCs using HS than DI, possibly due to their affinity with sample components adversely impacting their phase transition from a liquid to the gas phase during HS analysis, likely exacerbated by higher molecular weight VOCs. It is possible that the importance of lactones in many dairy products may have been underestimated due to the widespread use of HS-SPME DVB/CAR/PDMS and CAR/PDMS phases, where the CAR component may exclude some higher molecular weight lactones [29]. Differences in the apparent capacities of the phases associated with the different techniques did not have as much of an impact on VOC extraction as the difference between DI and HS. Differences between dynamic HS (TD) and static HS (HS-HiSorb and HS-SPME) techniques also did not significantly influence VOC extraction in terms of numbers and abundance. The reproducibility of most of the techniques, as assessed by the average relative percentage deviation, were similar, apart from HS-HiSorb, independent of salting out using the polar GC column, which was much diminished. However, reproducibility was very much VOC-dependent and also influenced by salting out and GC column polarity. Thus, in this study, differences between the techniques were impacted more by the choice of DI or HS, phase composition, and GC column polarity than phase capacity or salting out.

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REVIEW

Sensorial, cultural and volatile properties of milk, dairy powders, yoghurt and butter: A review

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Countries with an established dairy tradition consume milk, milk powder, yoghurt and butter directly or as an ingredient; however, in countries without this tradition the lack of familiarity and unknown expectations can be challenging to overcome. Therefore, having a better understanding of the volatile properties that influence their sensory appeal can aid overcoming these challenges. This review focusses on traditional and novel sensory methods used to research milk, milk powders, yoghurt and butter as well as the extraction techniques used in gas chromatography mass spectrometry and gas chromatography olfactometry to identify volatiles in these products that influence sensory perception.

Keywords Milk, Dairy powders, Yoghurt, Butter, Sensory, Volatile organic compounds.

INTRODUCTION

Sensory analysis is an important part of dairy product development and manufacture, providing answers to specific flavour, visual and textural characteristics and hedonic consumer responses amongst others. Affective tests incorporating preference and hedonic testing use subjective criteria of untrained consumers to provide important market information cost effectively (Stone et al. 2020). Combinations of affective and analytical techniques (threshold, discrimination and descriptive tests) are applied to take advantage of each technique's convenience for specific purposes providing important sensory information that can be used for example to improve product quality and/or market share. New sensory methodologies have been developed with the aim of rapidly providing sensory data more cost effectively, but doing so relatively simply in comparison to traditional techniques (Ruiz-Capillas et al. 2021). Such sensory methods include check-all-that-apply (CATA), flash profiling (FP) and rate-all-that-apply using trained panels or semi-trained panels.

Cross-cultural sensory and consumer research is becoming increasingly important, as it involves both consumer psychology and the dynamic interaction between the consumer, the context and the food (Lee et al. 2010). Culture is one of the significant factors underlying consumers' food choices, influencing attitudes and beliefs about food (Rozin 1988). Different food environments and dietary experiences across cultures influence both sensory perception and consumer preferences (Prescott and Bell 1995). Cross-cultural studies aid in the understanding of how consumers from different cultures perceive foods and assists in achieving market penetration, especially for new products or for unfamiliar products in new markets (Ares 2018).

As food aroma is such a significant factor in flavour, it is a widely researched topic, with over 10 000 volatile organic compounds (VOC) known to exist with less than 3% thought to contribute to the aroma of any given food (Dunkel et al. 2014). VOCs must be present at a

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concentration above their odour threshold in order to be perceived, this is typically measured as their odour activity value (OAV), which is the ratio of the concentration to the odour threshold. In most cases, the presence of multiple VOCs is essential for the characteristic aroma of a product, rather than a single VOC. It is also thought that many other factors not just the OAV of VOCs impact sensory perception, such as gustatory and trigeminal components and genetic differences between individuals (Spence 2021). However, understanding the VOC profile of a food gives an important insight into the relationship between VOC and multisensory flavour perception, but determining the true VOC profile of any product is difficult due to the many factors that can impact analysis.

A key aspect of VOC research that is often not addressed in dairy research is the actual relationship between VOC and sensory perception. This can be achieved to some extent using multivariate statistical analysis of VOC and sensory data (ideally descriptive sensory data) where some plausible associations can be implied; however, it is much more beneficial to undertake gas chromatography-olfactometry (GC-O) where individual aroma active VOC can be identified (Cadwallader and Singh 2009; Sarhir et al. 2021) as well as their potential significance to the overall aroma. There are several factors involved in the processing of dairy products that impact VOC; such as oxidative stability, thermal treatments, high pressure, ultrasound and addition of processing aids, ingredients, cultures or enzymes (Vazquez-Landaverde et al. 2005; Cadwallader and Singh 2009; Riener et al. 2009; Serra et al. 2009; Liu et al. 2022). Table 1 summarizes the composition and common thermal treatments applied to, milk, dairy powders, butter and yoghurt during processing.This review will focus on the key aroma active VOC in milk, dairy powders, yoghurt and butter and their relationship to product quality from a flavour perspective, incorporating cross cultural sensory analysis and new trends in sensory science applicable to these products. This review does not include cheese due to the added complexity of the product, and the fact that so many studies have been undertaken that it requires a separate independent review.

SENSORY ANALYSIS

Sensory techniques

Sensory science is used to assess, study and explain the response of the peculiarities of food that are observed by panellists using their senses of sight, smell, taste, touch and hearing (Stone et al. 2020). Sensory analysis is used to obtain a better understanding of the relationship between aroma and sensory perception. Different types of sensory analyses, from conventional methods (Consumer Acceptance Testing and Quantitative Descriptive Analysis) to novel rapid sensory techniques (Check All That Apply, Flash Profile, Temporal Dominance of Sensations, etc.) are used to understand more about key sensory attributes and/or preferences of dairy products (Drake 2007; Andrewes et al. 2021).

Consumer acceptance testing is easy to preform using hedonic scales without sensory training. The hedonic scale assumes that participants' preferences exist on a continuum and that their responses can be categorized into the degree of liking or disliking of sensory attributes, such as appearance, odour, taste, aroma, texture (O'Sullivan 2016). The most widely used scale for measuring food acceptability is the nine-point hedonic scale, which has ruler-like and equal-interval properties with 'dislike extremely' on the left and 'like extremely' on the right (Wichchukit and O'Mahony 2015). Previous sensory studies have employed between 18 and 310 consumers for hedonic testing of milk, butter, yogurt and dairy powders (Hoppert et al. 2013; Potts et al. 2017; Cheng et al. 2020; Garvey et al. 2020; Clarke et al. 2020a; da Silva et al. 2021).

Descriptive tests consist of a full sensory description of the products and require fewer panellists, but the panellists must be highly trained to distinguish between attributes previously selected through focus groups (using selected sensory attributes from product references or standards) that best describe the product, and to evaluate their perception with quantitative values (O'Sullivan 2016). Quantitative descriptive sensory analysis is one of main descriptive analysis techniques in sensory evaluation. Clarke et al. (2020b) used 12 trained (60 h) descriptive sensory panellists to assess milk samples from different diets and the results of full descriptive sensory analysis provided a reliable insight into the differences of milks based on cows feeding system. However, operating traditional descriptive trained panels is expensive and time consuming, and therefore other methods have been developed in order to obtain sufficient sensory information, but more rapidly and cost effectively.

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). Cheng et al. (2020) used ODP method to identify the sensory attributes of skim milk powder (SMP) produced from different cows diets with trained assessors from Ireland and China. Irish and Chinese trained assessors had different preferences for many attributes, and both found it more difficult to discern differences between SMP derived from cows outdoors fed perennial ryegrass or perennial ryegrass with white clover, than SMP produced from cows indoors on a concentrate diet.

CATA is another sensory approach to rapidly assess products. Consumers are presented with the sample and a versatile multiple-choice questionnaire, then asked to indicate which words or phrases appropriately describe their sensory experience (Ares et al. 2015). The terms might include sensory attributes, hedonic responses, emotional responses, purchase intentions, potential applications, product positioning

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Table 1 Summary of the processing method and compositional in milk, milk powder, yogurt and butter

Dairy products	Processing method	Typical thermal treatments	Compositional information (%)	References
Milk	UHT	135°C for 2–5 s		a
	High temperature short time (HTST)	72°C for 15 s	Moisture: 86; protein: 4.2; Fat: 3–4.6	b,c,d
	Low temperature long time (LTLT)	63°C for 30 min		d
	High hydrostatic pressure processing (HPP)	40°C, 600 MPa for 5 min		d
	Ultrasound (US)	400 W, 45°C for 2.5–20 min		e
SMP	Low heat spray drying	71°C for 20 min	Moisture: 3–4; protein: 34–37; Fat: 1.2	f,g
	Medium heat spray drying	71–79°C for 20 min		
	High heat spray drying	90°C for 30 min		
WMP	Medium-heat	65°C for 20 min	Moisture: 3–4; protein: 24–27; Fat: 26–29	h,i
Yogurt	High temperature short time (HTST)	72°C for 15 s	Moisture: 88; protein: 3–5; Fat: 3–4	j,k
	Ultra-high pressure homogenized (UHPH)	300 MPa, 90°C for 90 s		
Butter	High temperature short time (HTST)	72°C for 15 s	Moisture: 15; protein: 1; Fat: 80–83	l

The data adapted from (a) Vazquez-Landaverde et al. (2005), (b) Faulkner et al. (2018), (c) O'Callaghan et al. (2019), (d) Liu et al. (2020a), (e) Riener et al. (2009), (f) Karagül-Yüceer et al. (2001), (g) Turner et al. (2002), (h) Clarke et al. (2020b), (i) Lloyd et al. (2009), (j) Tian et al. (2017), (k) Serra et al. (2009), (l) O'Callaghan et al. (2016).

or other terms that the consumer might associate with the sample. Harwood and Drake (2020) used a list of 22 features in a CATA format to identify what features typically influenced panellists purchase of milk. The results demonstrated that consumers generally expressed preferences that aligned with their explicit beliefs, and flavour considerations appeared to be a secondary differentiator of preference.

FP is another rapid low cost technique where untrained panellists select their own terms to describe and evaluate a set of products simultaneously, and then rank the products for each attribute that they individually create. Panellists are forced to generate discriminative attributes of the whole sample set but not on a hedonic term (Delarue 2015). Yao et al. (2018) used FP with 17 sensory attributes developed by 10 panellists for yoghurts produced by pasteurisation or by thermisation. FP was able to discriminate yoghurts based on the heat-treatment applied.

Temporal dominance of sensations (TDS) is dynamic descriptive sensory technique that involves repeatedly assessing, until the sensations end, and determining which sensation is dominant in scoring its intensity (Pineau et al. 2009). Compared to time intensity, this method considers the multidimensionality of the perceptual space over time. Hutchings et al. (2017) used TDS to analysis milk protein hydrolysates using 20 consumers over six training sessions. Similar TDS results were obtained by the panellists from three levels of training session (untrained, familiarized and trained) for each product, but training also increased panel consensus and the ability to discriminate between milk protein hydrolysates. As the training session increased, the number of attributes selected decreased and the time spent on a given attribute increased.

Cross-cultural sensory analysis

The familiarity of food products plays an important role in acceptability and preference because it decreases the uncertainty about the safety and suspense associated with a novel product by generating a better match between expectations and sensory characteristics (Methven et al. 2012; Borgogno et al. 2015). For several studies, familiarity has had a positive effect on the liking scores of the food items and demonstrates a products' palatability and safety (Prescott 1998; Torrico et al. 2019). Liem et al. (2016) noted that Chinese consumers who had repeated exposure to the taste of ultra-high temperature (UHT) milk preferred UHT milk over pasteurised milk, highlighting that familiarity is a powerful driver of consumer liking. Cross-cultural differences exist in that familiarity may even influence trained panellists' perception of an attribute, e.g to be more or less intense than it actually is when responding to unfamiliar products (Lee et al. 2010). Tu et al. (2010) also concluded that the French panels who were less familiar with soya yoghurts needed twice as many attributes to describe the product's aroma than a Vietnamese panel who were more familiar with these products. Garvey et al. (2020) investigated the liking and perception of salted butters, produced from milk derived from different diets (perennial ryegrass or perennial ryegrass and white clover, or concentrate) by consumers in Ireland, Germany and the USA. The results demonstrated that familiarity contributed to sensory differences in Irish butter identified by German, Irish and USA consumers and assessors. Irish consumers preferred the appearance and flavour of butters produced from milk derived from cows outdoors on perennial ryegrass or perennial ryegrass and white clover, than German and USA

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consumers. German consumers found the salt intensity highest in butter produced from cows milk derived from the perennial ryegrass or perennial ryegrass and white clover, which was thought to relate to the softer texture of these butters and their more rapid melting properties due to changes in fatty acid content, as the salt contents were similar. Familiarity was also postulated to contribute to differences in 'appearance liking' and 'colour liking' of these butters by USA consumers, where the butter produced from milk derived from cows indoors fed on concentrate scored highest, as this is the most widespread type of this butter available in the USA.

Consumers may also rely on their memory associative structure created from past personal experiences to influence acceptance, which is heavily influenced by culture. The multidimensional experience (sensory perception, memory, culture and emotions) by consumers may increase acceptance for products (Corredor et al. 2010). The appearance is the first attribute evaluated by consumers and the visual information of the samples strongly influences the hedonic scores (Zampini et al. 2007). Satisfaction of these extrinsic aspects can influence overall liking, and thus purchase intent and even willingness to pay a premium, particularly for dairy products (Bir et al. 2020; Scozzafava et al. 2020). Hay et al. (2021) investigated consumer sensory preferences for drinkable yoghurt and the impact of provenance using Chinese, European and New Zealand consumers. In terms of sensory drivers 'sweetness', 'sourness', 'strawberry flavour', 'dairy flavour', 'creamy flavour' and 'creamy texture' and 'thickness' were correlated with culture. Chinese consumers had a cultural expectation for higher levels of sweetness compared to New Zealand and European consumers, while New Zealand consumers expected higher level of sourness, but not too sour. Dairy flavour was an important sensory attribute for Chinese consumers, and expectations concerning 'strawberry flavour' and 'thickness' also differed between the cultural groups.

Novel or unfamiliar food products are usually rejected by consumers and consistently score lower liking scores for all sensory attributes regardless of the cultural group (Pingali 2007). Tan et al. (2015) contrasted two groups of potential consumers with and without cultural exposure to specific foods and found that rejection of unfamiliar foods was greater than familiar foods, which can be considered a big factor in product development of novel food items. Ethnic food in a cultural community is often regarded as novel food by another community (Bell et al. 2011). Cheng et al. (2020) assessed consumer perceptions of SMP produced from milk derived from cows outdoors fed perennial ryegrass, or perennial ryegrass and white clover, or cows indoors fed concentrate in Ireland, China and USA. Chinese consumers could not discern a difference between the three SMP produced from the different diets, but rated 'aftertaste liking' and 'aftertaste intensity' differently than Irish and USA consumers, which may relate to the fact that some attributes were difficult to categorise with ambiguous cultural meanings. Moreover, Chinese consumers and trained assessors scored many attributes quite differently than their Irish or USA counterparts, likely again reflecting a lack of familiarity with dairy products. USA consumers had preference for SMP produced from milk derived from cows on a concentrate diet, while Irish consumers generally preferred SMP produced from cows on a pasture diet (either perennial ryegrass or perennial ryegrass and white clover diets), which reflects the main sources of cow diet used in both geographical locations.

Situational interpretations and meanings can also differ across languages and cultures. This can be a problematic for panels (consumers) only measuring the momentarily blinded sensory perception for preference, liking and acceptance, by the fact that anchors have also been shown to influence cultural differences (Yeh et al. 1998; Ares 2018). Sensory attributes do not necessarily have a direct relationship with a single ingredient and have not a direct translation across languages, and therefore can cause problems for consumers with dissimilar cultures and languages (Prescott 1998). Cheng et al. (2020) suggested that differences in the 'aftertaste liking' attribute for Chinese consumers in relation to their perception of SMP may have more to do with the verbalisation of sensory perception and linguistic representation, rather than the Western definition of the term. A similar result was found by Zhi et al. (2016), where a high 'aftertaste intensity of thickness and sweetness' is often used as a positive term to describe better quality milk in China and thus the concept of 'aftertaste' may be cultural dependent, because the underlying conceptual elements and words used to describe its features may be dissimilar. Pingali (2007) identified that creamy attributes would not be considered a common descriptor to delineate the characteristics of dairy products in the Chinese and Korean language. Chinese and Korean groups would use goso/xiāng (fragrancy) instead to describe their perception of dairy products.

It is necessary to validate scales (especially the meaning and psychological properties of scale labels) and any questions within the cultures of interest properly before conducting any cross-cultural sensory evaluation. Instructions to participants and questions should be accurately translated from one language to the other by a bilingual person to ensure that they hold the same meaning across all the cultural groups under consideration and to minimize differences in cultural interpretation and familiarity of any words (Helms 1992; Arnold and Smith 2013). Preference mapping can potentially allow the interpretation of preference data from another culture to be related to trained panel descriptions and measurements conducted in one's own language (Prescott 1998). Ares (2018) also suggested that the behavioural measurements such as the Ranking or Best–Worst

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scaling becomes an alternative to hedonic scaling, which could decrease the mistranslation in scale-usages styles/response styles between Asian and Western consumers. Lee and Lopetcharat (2017) highlighted that using a combination of behavioural measurements and sensometrics improved both the validity and reliability of cross-cultural sensory and consumer studies by both stabilizing the subjects' evaluative process and quantifying the effects of cultures. Kim et al. (2018) processed the verbal definition in conceptual elements of nutty with a sensory approach that correlates structured sensory space with cross-cultural sensory elements driving nuttiness perception. Their results revealed that each cultural group (Korean, Chinese and English-speaking-Western consumers) evaluated nuttiness in soymilk based on similar criteria, which avoided misunderstandings in sensory attributes caused by conceptual differences across culture. Köster and Mojet (2015) recommended the use of non-verbal methods, such as PrEmo (a tool used to measure the emotions evoked by materials), in cross-cultural research in order to overcome language differences in the use of emotional terms.

VOLATILE PROFILING BY GAS CHROMATOGRAPHY MASS SPECTROMETRY

Volatile extraction techniques

The VOC profile of dairy products can be influenced by animal diet, heat treatments, processing and storage conditions (Baldwin et al. 1991; Birchal et al. 2005; Kilcawley et al. 2018). As the composition of the dairy products varies extensively, this can have a significant impact on VOC extraction due to differences in VOC solubility in polar and non-polar phases within the product, and from interferences from other elements present, especially salts. These factors need to be taken into account to determine the most suitable method of extraction for their isolation and subsequent analysis (Jeleń et al. 2012). Many dairy products also contain active microbes that are dynamically undergoing enzymatic and or chemical changes that both directly and indirectly impact on the VOC profile.

A wide array of extraction techniques have been employed to isolate and concentrate VOCs from different dairy products, including, for example, solid-phase microextraction (SPME; Coppa et al. 2011; Clarke et al. 2019; Cheng et al. 2020), solvent-assisted flavour evaporation (SAFE; Evans et al. 2009), dynamic headspace extraction (DE; Ciccioli et al. 2004), thermal desorption (TD; Faulkner et al. 2018), stir bar sorptive extraction (SBSE; Schiano et al. 2019) and simultaneous distillation extraction (SDE; Kobayashi et al. 2008). However, reliable detection and complete quantification of VOCs in dairy products remains challenging (Schiano et al. 2019), as every technique has a degree of bias towards the extraction of certain chemical classes based aspects of the process itself, such as type of solvent or sorbent phase used (Ning et al. 2011). Therefore, it is best to utilise multiple extraction techniques if possible in an attempt to get the as true a volatile profile as possible for untargeted analysis. Most volatile extraction techniques are used in tandem with gas chromatography mass spectrometry (GC-MS), although other options exist, such as GC-FID (flame ion detection), SIFT-MS (selected ion flow tube mass spectrometry) and PTR−MS (proton-transfer reaction mass spectrometry) (Mariaca and Bosset 1997; Aprea et al. 2009; Olivares et al. 2011).

Microextraction methods that have a minimal amount of extractant phase enable fast sample preparation, high sensitivity and are more easily automatable, and are thus becoming more widely favoured for VOC characterization. In addition as 'green chemistry' techniques that are seen as more environmentally friendly which require little or no solvents are becoming increasingly favoured. Figure 1 (a) shows the results of Web of Knowledge search for extraction methods used in milk, SMP, whole milk powder (WMP), yoghurt and butter between 2000 and 2021. In total, ~44 publications were identified, dominated by headspace solid-phase microextraction (HS-SPME), with solvent-assisted flavour evaporation (SAFE) also widely used (Figure 1a). When looking into the types of dairy products in which multiple extraction techniques were used for analysis of VOC, the biggest group was for milk, followed by SMP, WMP, then butter and finally yoghurt (Figure 1b).

A summary of the volatiles identified in milk, dairy powders, butter and yoghurt from four common extraction methods by GC-MS are provided in Table 2. A total of 303 VOCs were identified by various extraction methods including alcohols (59), aldehydes (50), esters (38), ketones (30), organic acids (23), lactones (20), terpenoid compounds (17), carbonyl compounds (14), sulphur compounds (8) and furans (8).

Solid-phase microextraction

SPME is a widely used extraction technique, in part due to its relative simplicity (no extensive sample preparation) when compared to other techniques such as dynamic headspace extraction or solvent extraction. It is a manual or fully automotive technique (in conjunction with a robotic autosampler) and offers high reproducibility and is relatively inexpensive as SPME fibres can be used multiple times. SPME can be performed as a direct immersion procedure (DI-SPME) by exposing a phase-coated fibre into a liquid sample (Mallia et al. 2005), or as a headspace procedure (HS-SPME; Pawliszyn 1997; Januszkiewicz et al. 2008). DI-SPME is not that widely practiced for dairy products, due to the fact that it can adversely impact on the longevity of the fibres due to repeated swelling and drying, and fouling of the fibre can also occur which also adversely impacts on the ability of low-molecular-weight VOCs interaction with fibre phases (Heaven and Nash 2012). A main

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advantage of SPME is that a wide range of sorbent phases are available; from single phases; polydimethylsiloxane (PDMS) and polyacrylate (PA); dual phases; carboxen (CAR)/PDMS, PDMS/divinylbenzene (DVB), carbowax polyethylene glycol (CW-PEG), CW/DVB, CW/TR (templated resin), or as triple phases; DVB/CAR/PDMS (Mondello et al. 2005; Yu et al. 2008; Heaven and Nash 2012; Jeleń et al. 2012). The type of fibre coating and thickness determine the properties in terms of polarity and retention, which affects the extraction efficiency, selectivity, reproducibility and discrimination of the extraction (Spietelun et al. 2010). A range of film thicknesses are also available; PDMS at 100, 30 and 7 µm, PA at 85 µm, PDMS/DVB at 65 and 60 µm, CAR/PDMS or CW/DVB at 75 and 65 µm and CW/TR at 50 µm.

The most convenient way to discuss the coating capacity independent of its characteristics as a solid or liquid sorbent is to consider the fibre constant as defined by Rivellino et al. (2013), where

$$Fc = KfsVf$$

Fc = fiber constant, Kfs = the, fiber coating/sample matrix distribution constant of the analyte, Vf = volume of the extraction phase.

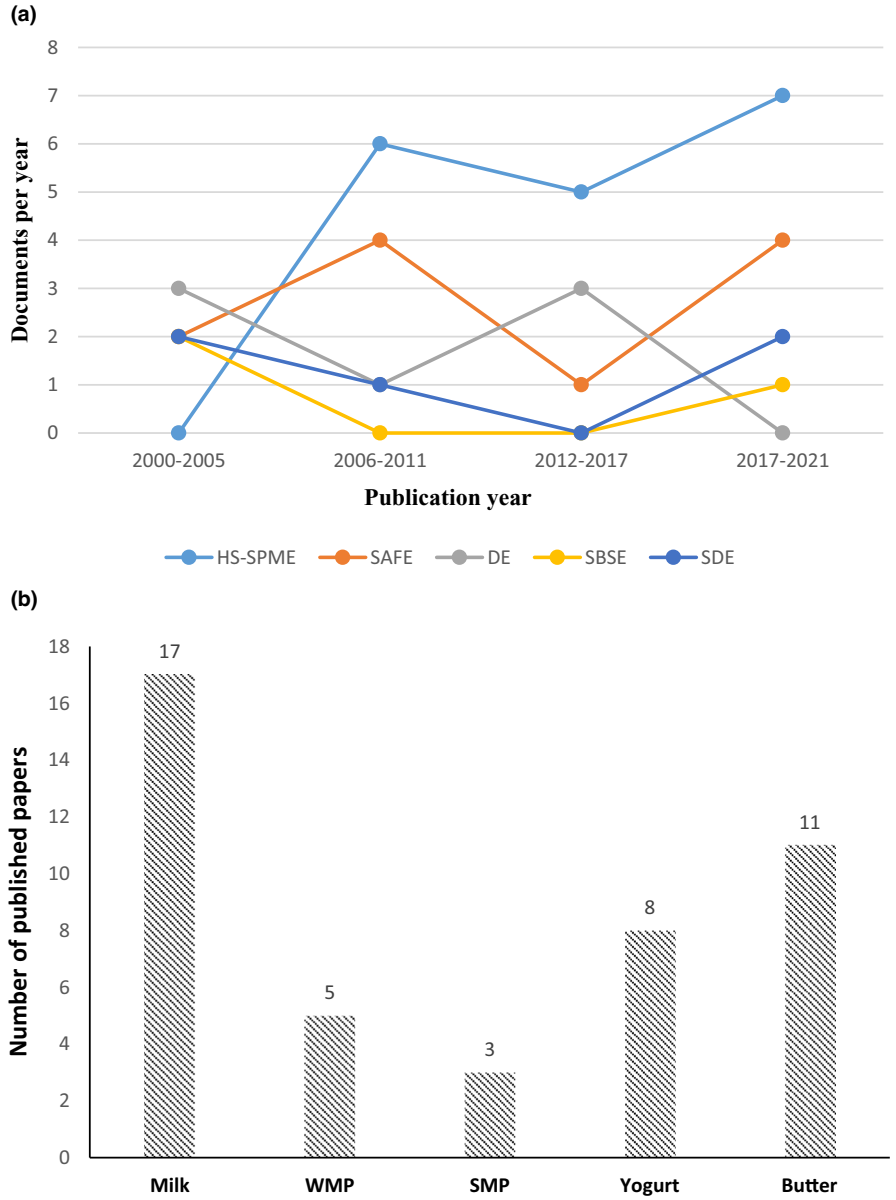

Figure 1 Applications of microextraction methods in selected dairy products. (a) Application of all extraction methods (HS-SPME, SAFE, DE, SBSE and SDE) used (b). Number of published studies based on Web of Knowledge search for years 2000–2021 incorporating all extraction techniques

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Table 2 Volatile compounds found in milk (milk powders), butter, and yoghurt using GCMS by four common extraction methods

Compound	CAS no.	Extraction methods				Ref
		SPME	SAFE	DE	SBSE	
Alcohols						
(E)-2-Hexen-1-ol	928-95-0		✓			a
(E)-2-Nonen-1-ol	31502-14-4		✓			a
(E)-2-Octen-1-ol	18409-17-1		✓			a
(E)-2-Octenal	2548-87-0		✓			a
1,3-Butanediol	24621-61-2		✓			b
1,4-Butanediol	110-63-4	✓				c
1-Butanol	71-36-3	✓	✓	✓		a,d,e
1-Dodecanol	112-53-8	✓				f
1-Heptanol	111-70-6	✓	✓	✓		a,g,h,i
1-Hexadecanol	36653-82-4				✓	j
1-Hexanol	111-27-3	✓	✓	✓		a,e,i,j,l
1-Nonanol	143-08-8	✓	✓			a,i,k,l
1-Octadecanol	112-92-5		✓		✓	j,m
1-Octanol	111-87-5	✓	✓	✓	✓	a,h,i,j,l
1-Pentanol	71-41-0	✓	✓	✓		a,d,e,f,h,k,n,o
1-Penten-3-ol	616-25-1		✓			a
1-Phenylethanol	98-85-1				✓	j
1-Propanol	71-23-8		✓	✓		a,e,h
1-Tetradecanol	112-72-1				✓	j
2-(Methylthio)-ethanol	5271-38-5		✓			a
2,3-Butanediol	513-85-9	✓	✓			a,k
2-Butanol	78-92-2	✓	✓			b,i
2-Ethyl-1-hexanol	104-76-7	✓	✓			d,l
2-Furanmethanol	98-00-0	✓	✓	✓	✓	a,f,j,p,q
2-Heptanol	543-49-7		✓			b
2-Hexanol	626-93-7			✓		e
2-Methyl-1-butanol	137-32-6	✓				n
2-Methyl-1-propanol	78-83-1		✓	✓		a,e
2-Methyl-2-propanol	75-65-0			✓		e
2-Methyl-3-furanthiol	28588-74-1	✓	✓			q,r
2-Methyl-3-pentanol	565-67-3	✓				i
2-Nonanol	628-99-9	✓				i
2-Octanol	123-96-6	✓				f
2-Pentanol	6032-29-7		✓	✓		b,e
2-Phenethanol	60-12-8		✓			r
2-Phenoxyethanol	122-99-6		✓			b
2-Propanol	67-63-0	✓				i
3-Hexanol	623-37-0	✓		✓		h,i
3-Methyl-1-butanol	123-51-3		✓			a,b
3-Methyl-2-butanol	598-75-4	✓				b,k
3-Methyl-2-hexanol	2313-65-7		✓			b
3-Methyl-3-buten-1-ol	763-32-6	✓	✓			a,k
3-Octanol	589-98-0		✓			a
3-Pentanol	584-02-1	✓				k
3-Penten-2-ol	3899-34-1	✓				k
4-Methyl-1-pentanol	626-89-1	✓				k
4-Methyl-2-pentanol	108-11-2			✓		e
4-Methyl-2-pentanol	108-11-2		✓			b

(continued)

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Table 2 (Continued).

Compound	CAS no.	Extraction methods				Ref
		SPME	SAFE	DE	SBSE	
Benzyl alcohol	100-51-6	✓	✓			a,d
Ethanol	64-17-5	✓	✓	✓		d,h,k,s,t
Ethyl furaneol	27538-10-9	✓				u
Furaneol	3658-77-3		✓			q
Heptanol	111-70-6	✓				i
Hexanol	111-27-3	✓				c
Isomaltol	3420-59-5				✓	j
Maltol	118-71-8		✓			w
Methionol	505-10-2		✓			a
Phenethyl alcohol	60-12-8		✓			a,l
Tetradecanol	112-72-1		✓			r
Aldehydes						
(E)-2-Heptenal[a]	18829-55-5	✓	✓			a,f
(E)-2-Hexanal	6728-26-3		✓			y
(E)-2-Nonenal	18829-56-6	✓	✓	✓	✓	a,g,j,r,w,x
(E)-2-Octenal	2548-87-0		✓			w
(E)-2-Undecenal	53448-07-0		✓			r
(E,E)-2,4-Decadienal	25152-84-5		✓			a,r
(E,E)-2,4-Heptdienal	4313-03-5	✓	✓			a,f,m
(E,E)-2,4-Nonadienal	5910-87-2	✓	✓			f,r
(E,Z)-2,4-Decadienal	25152-83-4		✓			r
(E,Z)-2,4-Nonadienal	5910-87-2		✓			m,w
(E,Z)-2,6-Nonadienal	557-48-2		✓			a,r,w,x
(Z)-2-Decenal	2497-25-8				✓	j
(Z)-2-Nonenal	60784-31-8		✓			w,x
(Z)-3-Hexenal	6789-80-6		✓			y
(Z)-4-Heptenal	6728-31-0		✓			m,r,w,x,y,δ
2,4-Decadienal[a]	2363-88-4	✓		✓		g,p
2,4-Hexadienal[a]	142-83-6			✓		e
2,4-Nonadienal[a]	6750-03-4			✓		p
2-Decenal[a]	3913-71-1	✓	✓	✓		a,f,p
2-Dodecenal	4826-62-4		✓			r
2-Ethyl-4-pentenal	5204-80-8	✓				f
2-Heptenal[a]	18829-55-5			✓		p
2-Methyl-2-propenal	78-85-3			✓		e
2-Methyl-butanal	96-17-3	✓	✓	✓		n,r,x,y,z
2-Methyl-propanal	78-84-2			✓		h
2-Nonenal	18829-56-6			✓		p
2-Octenal	2363-89-5			✓		p
2-Undecenal	2463-77-6			✓		p
3-Methyl-butanal	590-86-3	✓	✓	✓		i,r,y,α
4-Ethyl-benzaldehyde	4748-78-1	✓				p,q
6-Decenal	147159-48-6	✓				f
Acetaldehyde	75-07-0	✓				i,k,η
Benzaldehyde	100-52-7	✓	✓	✓		b,c,d,h,i,n,o,s,t
Benzeneacetaldehyde	122-78-1	✓				f
Butanal	123-72-8	✓				I,c
Decanal	112-31-2	✓	✓	✓	✓	a,d,i,j,k,m,o,r,t,w,y,α
Dodecanal	112-54-9				✓	j
Heptanal	111-71-7	✓	✓	✓	✓	a,d,g,j,n,o,r,t,w,y,α

(continued)

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Table 2 (Continued).

Compound	CAS no.	Extraction methods				Ref
		SPME	SAFE	DE	SBSE	
Hexadecanal	629-80-1		✓			a
Hexanal	66-25-1	✓	✓	✓	✓	c,g,i,j,m,n,p,r,t,w,x,y,β,γ,δ
Methional	3268-49-3	✓	✓			r,s,w,y,δ
Nonanal	124-19-6	✓	✓	✓	✓	a,c,d,h,i,j,k,m,n,o,p,r,u,w,x,z,β,δ
Octanal	124-13-0	✓	✓	✓	✓	g,j,m,p,r,z,δ
Pentadecanal	2765-11-9		✓			a
Pentanal	110-62-3	✓	✓	✓	✓	d,g,j,n,r,t,α
Phenylacetaldehyde	122-78-1		✓			r
Pyruvaldehyde	78-98-8		✓			y
Tetradecanal	124-25-4	✓	✓			a,β
Tridecanal	10486-19-8		✓			δ
Undecanal	112-44-7	✓				g
Carbonyl compounds						
4-Ethylphenol	123-07-9		✓			m,y
Benzene	71-43-2	✓		✓		e,f
Ethyl benzene	100-41-4	✓		✓		g,h
Ethyl ether	60-29-7			✓		h
Guaiacol	90-05-1		✓			m,r,δ
m-Cresol	108-39-4		✓			b
m-Xylene	108-38-3	✓		✓		e,f,n
o-Xylene	1330-20-7	✓				n
Phenol	108-95-2	✓	✓			d,k,s,y
p-Xylene	106-42-3	✓				i
Styrene	100-42-5		✓			x
Toluene	108-88-3	✓		✓		c,d,e,h,f,k,n,t,z,α
p-Cresol	106-44-5	✓	✓		✓	f,w,y
Dehydro-p-cymene	1195-32-0		✓			c
Ketones						
1-Hexene-3-one	1629-60-3		✓			m,r,x
1-Hydroxy-2-acetone	116-09-6	✓	✓	✓		c,b,d,p
1-Nonen-3-one	24415-26-7		✓			r
1-Octen-3-one	4312-99-6		✓			w,x,y,δ
2,3-Pentanedione	600-14-6		✓			y
2-Aminoacetophenone	551-93-9		✓			m
2-Butanone	78-93-3	✓		✓		d,e,i,k,n,t,z,α
2-Decanone	693-54-9		✓			a
2-Heptanone	110-43-0	✓	✓	✓	✓	b,e,g,i,j,k,n,x,z,α,β
2-Hexadecanone	544-76-3		✓			a
2-Hexanone	591-78-6	✓		✓		e,k,z
2-Nonanone	821-55-6	✓	✓	✓		b,c,g,k,n,s,t,z,β
2-Octanone	111-13-7	✓	✓	✓		a,i,z
2-Pentadecanone	2345-28-0	✓		✓	✓	j,s
2-Pentanone	107-87-9	✓		✓		e,g,i,k,n,t,z,α
2-Tridecanone	593-08-8	✓				i,k,s,β
2-Undecanone	112-12-9	✓	✓			a,d,f,i,k,q,s,β
3-Hexanone	589-38-8			✓		e
3-Octanone	106-68-3		✓			o
3-Octen-2-one	1669-44-9	✓				g
3-Pentanone	96-22-0			✓		e
4-Decanone	624-16-8		✓			g

(continued)

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Table 2 (Continued).

Compound	CAS no.	Extraction methods				Ref
		SPME	SAFE	DE	SBSE	
4-Methyl-2-hexanone	105-42-0			✓		e
4-Methyl-2-pentanone (methyl isobutyl ketone)	108-10-1	✓	✓	✓		e,k,o
5-Methyl-2-hexanone	110-12-3			✓		e
Acetoin	513-86-0	✓	✓			a, b,c,d,i,k,y,β
Acetone	67-64-1	✓		✓		c,d,g,i,k,n,t,α
Acetophenone	98-86-2			✓	✓	h,j
2,3-Butanedione (diacetyl)	431-03-8	✓	✓			b,c,i,k,r,t,u,w,x,y,ε,η
Sulcatone	110-93-0	✓		✓		i,z
Lactones						
Geranylactone	689-67-8	✓				i
Sotolone	28664-35-9		✓			r
γ-Butyrolactone	96-48-0		✓			l
γ-Crotonolactone	497-23-4				✓	j
γ-Decalactone	706-14-9	✓	✓			w,y,β
γ-Dodecalactone	2305-05-7	✓	✓			w,y,β
γ-Hexadecalactone	695-06-7		✓			β
γ-Nonalactone	104-61-0	✓	✓			r,x,ε
γ-Octalactone	104-50-7	✓	✓			r,β
γ-Tetradecalactone	2721-23-5		✓			y
γ-Undecalactone	104-67-6		✓			a
ε-Caprolactone	502-44-3	✓				d
σ-Decalactone	706-14-9	✓	✓		✓	f,j,l,o,r,s,t,w,x,y,β,ε
σ-Dodecalactone	713-95-1	✓	✓		✓	a,b,d,f,j,m,o,q,β
σ-Hexalactone	695-06-7	✓	✓			o,t,w,y
σ-Octalactone	104-50-7	✓	✓			o,t,u,w,x,y
σ-Tetradecalactone	2721-22-4		✓			y
σ-Undecalactone	104-67-6		✓			w
σ-Valerolactone	542-28-9		✓		✓	j,l
5-Ethyl-2(5H)-furanone	2407-43-4	✓				d,z,β
Sulphur compounds						
Dimethyl sulfide	75-18-3	✓	✓			k,r,t,x,η
Dimethyl sulfoxide	67-68-5		✓	✓		a,z
Dimethyl trisulfide	3658-80-8	✓	✓	✓		q,r,w,x,y,z,δ
Dimethyl disulfide	624-92-0	✓	✓	✓		m,r,s,z,α,δ
Dimethyl sulfone	67-71-0	✓	✓			d,f,i,o,l
Dimethyl tetrasulfide	5756-24-1		✓			t,δ
Dipropyl disulfide	629-19-6	✓				q
2-Methylthiophene	554-14-3		✓			y
Terpenoid compounds						
2-Carene	554-61-0	✓				v
3-Carene	3466-78-9	✓		✓		d,e,v
4-Terpineol	562-74-3			✓		e
Camphene	79-92-5			✓		e
Limonene[a]	5989-27-5	✓	✓	✓		b,k,o,e,h,v,y,α,β,λ
p-Cymene	99-87-6	✓	✓	✓		f,o,v
Sabinene	3387-41-5			✓		e
Squalene	111-02-4				✓	j
Styrene	100-42-5			✓		z
Terpinolene	586-62-9	✓				v
α-Pinene	7785-70-8	✓	✓	✓		d,e,t,v

(continued)

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Table 2 (Continued).

Compound	CAS no.	Extraction methods				Ref
		SPME	SAFE	DE	SBSE	
α-Terpinene	99-86-5	✓		✓		e,v
α-Thujene	2867-05-2		✓	✓		e, y
β-Caryophyllene	87-44-5		✓			y
β-Pinene	127-91-3	✓	✓	✓		e,f,n,t,y
γ-Terpinene	99-85-4		✓			m
(E,E)-Farnesyl acetate	4128-17-0		✓			r
Esters						
2-Methyl-butyl-acetate	624-41-9		✓			r,δ
3-Phenylpropionate	2012-28-5	✓				k
Butyl acetate	123-86-4	✓		✓		k
Butyl benzoate	136-60-7	✓				a,b
Ethyl acetate	141-78-6	✓	✓	✓		c,h,n,t,x,y
Ethyl butanoate	105-54-4	✓	✓			b,f,r,x
Ethyl decanoate	110-38-3		✓			f
Ethyl heptanoate	106-30-9			✓		h
Ethyl hexadecanoate	628-97-7	✓	✓			a,β
Ethyl hexanoate	123-66-0		✓	✓		a,b,h
Ethyl lactate	97-64-3		✓			b
Ethyl nonanoate	123-29-5		✓	✓		a,h
Ethyl octanoate	106-32-1	✓	✓			a,b,n,r
Ethyl pentanoate	539-82-2			✓		h
Ethyl tetradecanoate	124-06-1	✓				β
Ethyl tridecanoate	28267-29-0		✓			a
Ethyl undecanoate	627-90-7			✓		h
Ethyl-2-hydroxy-3-methyl-butanoate	2441-06-7		✓			a
Ethyl-2-hydroxy-hexanoate	52089-55-1		✓			y
Ethyl-2-hydroxy-propanoate	687-47-8		✓			a
Ethyl-2-methyl-butyrate	7452-79-1		✓			y
Ethyl-3-hydroxly-butanoate	5405-41-4		✓			a
Ethyl-3-methyl-butyrate	108-64-5		✓			a
Ethyl-9-decenoate	67233-91-4		✓			a
Heptyl hexanoate	6378-65-0	✓				f
Hexyl acetate	142-92-7			✓		h
Isopropyl hexadecanoate	142-91-6				✓	j
Linalyl acetate	115-95-7		✓			l
Methyl butanoate	623-42-7		✓			μ
Methyl dodecanoate	111-82-0	✓				a
Methyl heptanoate	106-73-0			✓		h
Methyl hexadecanoate	112-39-0	✓			✓	f,j
Methyl isobutyrate	547-63-7			✓		h
Methyl octanoate	111-11-5	✓				f
Methyl tetradecanoate	124-10-7	✓				β
Phenylethyl acetate	103-45-7		✓			a,r
Diisobutyl phthalate	84-69-5	✓				F
Propyl benzoate	2315-68-6	✓				c
Furans						
2-Furan-methanol	98-00-0		✓			l
5-Hydroxymethyl-furfural	67-47-0				✓	j
5-Methyl furfural	620-02-0		✓			m
2,3-Dihydro-2methyl-benzofuran	1746-11-8	✓				f

(continued)

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Table 2 (Continued).

Compound	CAS no.	Extraction methods				Ref
		SPME	SAFE	DE	SBSE	
2-Methyl furan	534-22-5	✓				f
Furfural	98-01-1	✓	✓	✓	✓	a,c,j,p
Homofuraneol	27538-09-6		✓			m
Hydroxy-2(5)H-furanone	14032-66-7				✓	j
Acids						
2-Methylbutanoic acid	116-53-0		✓			l
2-Methylpropanoic acid	79-31-2		✓			a,b,l,w
3-Methylbutanoic acid	503-74-2		✓			b,l,w,x,y
4-Methyloctanoic acid	54947-74-9		✓			r
9-Decenoic acid	14436-32-9		✓			l,w
Acetic acid	64-19-7	✓	✓	✓	✓	a,b,c,d,h,i,j,k,m,n,q,r,s,w,x,y,ε,λ
Benzoic acid	65-85-0	✓	✓			c,f,k,l,j,β
Butanoic acid	107-92-6	✓	✓		✓	a,c,d,f,j,k,l,i,n,q,r,s,w,x,y,ε,λ,β
Cyclohexylcarboxylic acid	98-89-5		✓			l
Decanoic acid	334-48-5	✓	✓		✓	a,b,c,f,j,k,i,l,m,o,r,s,w,β,δ
Dodecanoic acid	143-07-7	✓	✓			a,f,l,o,w,β
Formic acid	64-18-6		✓			l,w
Heptanoic acid	111-14-8	✓	✓			a,b,c,k,l,w,i
Hexadecanoic acid	57-10-3	✓	✓			o,β
Hexanoic acid	142-62-1	✓	✓	✓	✓	a,b,c,f,j,k,l,m,n,p,s,t,x,β,ε
Lauric acid	143-07-7		✓			a
Nonanoic acid	112-05-0	✓	✓		✓	a,b,c,f,j,k,l,o,t,β,ε
Octanoic acid	124-07-2	✓	✓		✓	a,c,f,I,j,l,k,o,s,w
Pentanoic acid (Valeric acid)	109-52-4	✓	✓	✓		a,b,i,h,l,m,r,w,y
Phenylacetic acid	103-82-2		✓			l,y
Propanoic acid	79-09-4		✓			a,b,d,w,l
Tetradecanoic acid	544-63-8	✓	✓			a,f
Undecanoic acid	112-37-8	✓	✓			a,f,l,β

Abbreviation: DE, Dynamic extraction; SAFE, Solvent-assisted flavour evaporation; SBSE, Stir bar sorptive extraction; SPME, Solid-phase microextraction.

The data adapted from (a) Ning et al. (2011), (b) Sarhir et al. (2021), (c) Su et al. (2017), (d) Cheng et al. (2020), (e) Ciccioli et al. (2004), (f) Coppa et al. (2011), (g) Clarke et al. (2019), (h) Rabaud et al. (2003), (i) Tian et al. (2017), (j) Faulkner et al. (2018), (k) Tian et al. (2019), (l) Miyaji et al. (2021), (m) Smith et al. (2016), (n) O'Callaghan et al. (2016), (o) High et al. (2019), (p) Francesca et al. (2015), (q) Mallia et al. (2014), (r) Evans et al. (2010), (s) Dadali and Elmaci (2019), (t) Garvey et al. (2020), (u) Abilleira et al. (2010), (v) Mahajan et al. (2004), (w) Lozano et al. (2007), (x) Bendall (2001), (y) Jansson et al. (2014), (z) Contarini and Povolo (2002), (α) Sarrazin et al. (2011), (β) Panseri et al. (2011), (γ) Evans et al. (2009), (ε) Martin et al. (2011), (η) Salum and Erbay (2019), (λ) Guneser and Yuceer (2011).

[a]Compounds are unidentified isomers.

HS-SPME extraction is considered complete when the analyte concentration has reached a distribution equilibrium between the sample, headspace and the fibre (Mondello et al. 2005). The efficiency of adsorptive extraction is dependent on the analyte surface concentration in the extraction phase at equilibrium and the surface area of the extraction phase (Musteata and Pawliszyn 2005). During the extraction process, the volume of VOC absorbed by the fibre phase is much faster than its release from the matrix, thus the requirement for sufficient time to obtain a representative VOC profile (Zabaras and Wyllie 2001). The length of extraction time and temperature are critical for SPME extraction efficiency. Generally, longer extraction times and high temperatures benefit the equilibrium resulting in increased responses of less volatile analytes (Fang and Qian 2005). However, care must be taken not to lose, create or enhance some VOC by the application of thermal treatments. The selectivity of HS-SPME is impacted by the selectivity of different phases towards specific solutes and various degrees of polarities. For example, larger less volatile compounds are captured by the porous DVB phase, while lower molecular weight highly volatile compounds

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are captured by the porous CAR layer (Garcia-Esteban et al. 2004). DVB is composed of polymerized alkyl chains with phenyl groups creating a porous phase that is used in combination with PDMS to aid in attaching the DVB and to increase selectivity (Heaven and Nash 2012). The PDMS phase tends to capture low to medium polarity compounds, with PA more suitable for highly polar compounds (Mondello et al. 2005). PA fibres are made of partially cross-linked acrylic acid monomers and swell slightly in water (Heaven and Nash 2012). CAR consists of different sized pores that capture compounds and are used in combination with PDMS as this helps attach the CAR to the fibre but also enhances selectivity (Heaven and Nash 2012). CW/DVB has ability to extract a wide range of low- to mid-molecular-weight molecules (Carpino et al. 2004).

Merkle et al. (2015) mentioned that the binding of analytes to the matrix resulted in low concentrations of the analytes in the headspace in complex food matrices. Thus, the matrix effect is worth considering when developing a HS-SPME or any HS method for the extraction of VOC in dairy products. However, high temperatures during extraction can reduce the adsorption ability of SPME fibre for the target analytes because the adsorption of fibre is an exothermic process (Ng et al. 1999). Generally, longer extraction times and high temperatures benefits the equilibrium and increases the responses of less volatile analytes, but often at the cost of sensitivity and possibly increase artifact formation (Mariaca and Bosset 1997). However, the quantification of sulphur VOCs can only be achieved under non-equilibrium conditions using short extraction time, particularly for complex matrixes due to their inherent instability (Murray 2001; Nielsen and Jonsson 2002). In certain cases, low extraction efficiencies are reported, in particular for very volatile, polar or thermally unstable analytes (Namieśnik et al. 2000). This is likely related to the relatively low capacity of the sorbent phases on the fibre in comparison to many other sorbent type extraction techniques.

Most HS-SPME studies involving milk, yoghurt, butter or dairy powders have used the three phase fibres. DVB/CAR/PDMS is particularly useful for the detection of highly volatile sulphur, alcohol, terpenes, esters and acid compounds (Abilleira et al. 2010). However, the overall recovery of more polar compounds, especially free fatty acids by the DVB/CAR/PDMS fibre is poor (Mondello et al. 2005). Tian et al. (2017) used a 50-{m DVB/CAR/PDMS fibre to extract VOCs in yoghurt. These authors found that an extraction/equilibration time of 40 min at 55°C extracted 45 VOCs (aldehydes, alcohols, ketones, organic acids and sulphur compounds), with ketones and aldehydes the most abundant chemical classes, followed by alcohols, acids and sulphur compounds. Tian et al. (2019) subsequently extracted 54 VOC also in yoghurt samples using this same HS-SPME procedure (extraction time 40 min at 55°C) and fibre. Ketones, aldehydes and alkanes were the most abundant chemical classes followed by alcohols, acids, carbonyl compounds and sulphur compounds. O'Callaghan et al. (2016) investigated VOCs in sweet cream butter derived from cows milk using a 75-{m DVB/CAR/PDMS fibre. The butter was equilibrated at 40°C for 10 min, then the fibre was exposed to the headspace for a further 20 min at 40°C. In total, 25 VOC were extracted consisting of aldehydes, ketones, alcohols, acids, esters, a terpene and toluene, p-xylene and phenol. Garvey et al. (2020) investigated VOCs in salted butter using an optimised HS-SPME method with a 50/30 {m DVB/CAR/PDMS fibre, with a pre-equilibration of 10 min at 40°C, followed by a 60 min extraction time at 40°C. They identified 30 VOCs consisting of aldehydes, ketones, acids, hydrocarbons, lactones, sulphur compounds, esters, alkenes and a terpene and alcohol compound. This study highlighted that aldehydes, ketones, acids, terpenes and lactones were the main chemical classes contributing to the volatile profile of butter. Mallia et al. (2014) identified VOCs (aldehydes, ketones, acids, lactones, hydrocarbons, sulphur compounds and an alcohol) in sour cream butters from different countries also using a 50/30 {m DVB/CAR/PDMS fibre with an extraction temperature of 45°C for 45 min. Cheng et al. (2020) also used a 50/30 {m DVB/CAR/PDMS fibre to extract VOCs from SMP with an equilibration/extraction temperature of 40°C for 20 min. These authors extracted 26 VOCs (aldehydes, ketones, alcohols, terpenes, acids, a sulphur compound and a phenyl compound). Cheng et al. (2021) found the HS-SPME with DVB/CAR/PDMS appeared to be very effective at recovering terpenes and sulphur compounds in WMP, but much less effective at recovering lactones, furans and acids.

Clarke et al. (2019) optimized the extraction of VOCs associated with lipid oxidation in WMP (2.4 g made up with 3.5 mL distilled water). The authors used the 50/30 {m DVB/CAR/PDMS fibre and found that an extraction time of 45 min at 43°C using 2.4 g of sample gave achieved the best extraction efficiency for VOC recovery. For the vast majority of the VOC selected (aldehydes and ketones), the limits of detection (LOD) varied between 0.002 and 0.006 mg/L, with limits of quantification (LOQ) of 0.05 and 0.066 mg/L. The authors noted a matrix effect, which was due to the degree of interactions of VOC with the sample, which was more apparent for longer chain aldehydes, likely due to their affinity with the fat phase in the WMP. The authors also concluded that the influence of the sample amount is less important for the recovery of polar than for non-polar VOCs. Matrix interference is a major issue with VOC analysis in foods, but especially for lipophilic compounds (Abilleira et al. 2010). One main reason is that as the solubility of VOCs increases in a hydrophobic solvent, while the vapour-liquid partition coefficient decreases (Druaux et al. 1998). Abilleira et al. (2010) also utilised the 50/30 {m DVB/CAR/PDMS fibre to quantify terpenes in ewe's milk fat, using a pre-equilibration time of 10 min at 40°C,

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followed by extraction at 40°C for 30 min. The authors noted that the matrix effect was a main reason for the overall systematic error to quantify terpenes (mono- and sesquiterpenes) in milk fat by HS-SPME.

Coppa et al. (2011) extracted VOC from the cream of cows milk derived from a hay-based diet or from continuous grazing of pasture. They also used the DVB/CAR/PDMS fibre. In this study, the cream was frozen and thawed in a HS vial at 60°C for 20 min in a water bath, and then incubated with the fibre exposed at 60°C for a further 20 min. Seventy-five VOCs were identified, and the study demonstrated that the DVB/CAR/PDMS fibre recovered VOCs with both high and low polarities. However, the relatively high temperature (60°C) used in this study, may induce artifact formation, or result in the higher abundance of some VOC (Mariaca and Bosset 1997). Dursun et al. (2017) used HS-SPME with 50/30 {m DVB/CAR/PDMS fibre at 55°C for 30 min to extract VOC from UHT milk to determine the correlations between individual aroma VOC and flavour attributes in UHT milks stored at the same conditions. A total of 43 VOCs (aldehydes, alcohols, ketones, acids, aromatic hydrocarbons, nitrogenous, sulphur containing compounds and an alkane hydrocarbon) were identified. The temperature of extraction (55°C) may again have resulted in increased abundance of some VOC or even artifact formation.

The bipolar CAR/PDMS has also been used extensively to extract VOCs from many dairy products, and it is particularly sensitive for the extraction of low-molecular-weight polar/apolar analytes (up until C6–C8) because of its porosity and the characteristics of its micropores (Shirey 2000; Mondello et al. 2005). The DVB-coated fibres contain relatively few micropores and CAR-coated ones contain a wide range of pores (micro-, meso- and macro-) in similar volumes (Elmore et al. 2000). Studies have shown that CAR/PDMS is very effective for the analysis of lower boiling point VOCs (Elmore et al. 2000; Januszkiewicz et al. 2008). Salum et al. (2017) optimized and compared the efficiency of DVB/CAR/PDMS and CAR/PDMS fibres for the extraction of specific VOC (3-methyl-1-butanol, ethyl lactate, 2-nonanone, ethyl octanoate, 2-ethyl-1-hexanol, butanoic acid, phenethyl alcohol, phenol, δ-decalactone and decanoic acid) in white-brined cheese. These authors found that optimum conditions for the CAR/PDMS fibre were 56.2°C for 84.92 min, slightly different to that for the DVB/CAR/PDMS fibre at 54.75°C for 85.60 min.

Other studies have shown that the CAR/PDMS fiber was more suitable for the extraction of low-molecular-weight VOCs such as 3-methyl butan-1-ol, ethyl lactate and butanoic acid and increasing the extraction time resulted in an increase in the volume of extracted VOCs for CAR/PDMS (Trujillo-Rodríguez et al. 2014). Martin et al. (2011) investigated the effect of oxidoreduction potential (Eh) on the biosynthesis of aroma compounds in non-fat yoghurt by HS-SPME using a 75-{m CAR/PDMS fibre for 40 min extraction time at 50°C. These authors demonstrated that the CAR/PDMS fibre was very sensitive for the extraction of acetaldehyde, dimethyl sulphide, 2,3-butanedione and 2,3-pentanedione. Su et al. (2017) also used the CAR/PDMS fibre to evaluate the VOC profile in yoghurt for 30 min at 60°C. They identified 30 VOC mainly consisting of aldehydes, ketones and acids plus some alcohols and esters; however, the increased temperature of extraction may have inadvertently enhanced the abundance of some VOC or even created new ones. Panseri et al. (2011) developed and validated a HS-SPME GC-MS method to quantify hexanal in butter to monitor lipid oxidation. They used an 85 {m CAR/PDMS fibre at 4°C for 180 min, and the low temperature was selected to minimise matrix oxidation and hexanal production during sampling. The results showed that CAR/PDMS fibre was especially sensitive to small molecules and suitable to monitor hexanal content both in fresh and oxidised butter samples.

SPME-Arrow has been developed to overcome the capacity limitation of traditional SPME as it has 6 to 20 times more volume capacity (Kim et al. 2020), but is also much less fragile. Manousi et al. (2020) compared a range of traditional SPME fibres (PDMS 100 {m), CAR/PDMS (75 {m), DVB/PDMS (65 {m) to SPME Arrow fibres (PDMS 100 {m), CAR/PDMS (120 {m) and DVB/PDMS (120 {m). These authors found that using CAR/PDMS SPME-Arrow out preformed their equivalent traditional fibre type by 4 or 5 times in terms of recovery using optimised conditions of 50°C for 60 min without salting out for 5 mL milk, but it was VOC dependent. However, to date very little studies have been published on SPME-Arrow on dairy products. In addition, another new SPME technique, thin film solid phase microextraction (TF-SPME), has been developed that has a very different geometry (a flat planar surface), that effectively increases the surface area-to-volume ratio and thus avoiding the usual caveats of increased phase volume (Bruheim et al. 2003). The simultaneous increase of extraction phase volume and surface area for TF-SPME (CAR/PDMS and DVB/PDMS) devices increases the potential for enhanced sensitivity with as good or better extraction rates compared to traditional SPME fibre (PDMS/DVB; Emmons et al. 2019); however, to date no studies in relation to dairy products appear to have been published.

Stir bar sorptive extraction

Stir bar sorptive extraction (SBSE) is another virtually solventless sample extraction technique available with two coatings (PDMS and PDMS with polyethylene glycol-modified silicone) of varying thickness (Ochiai et al. 2013). SBSE uses a small magnetic stir bar encased in glass and coated in sorbent material to detect the organic compounds. The principle of SBSE is based on the sorption of VOCs in an aqueous solution or semi-liquid matrix. A major advantage of SBSE is its high sensitivity towards semi-volatiles (Jeleń et al. 2012). The most widely used sorptive

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extraction phase is PDMS. The choice of extraction coating is a key factor that determines the extraction performance, in terms of extraction efficiency, selectivity and dynamics. PDMS is a commonly used coating for SBSE, and it has a good adsorption performance for analytes with weak polarity through hydrophobic force (Fan et al. 2020). The amount of coating (PDMS) in SBSE is usually 50–250 times larger than traditional SPME with 1 cm length × 0.5 mm or 2 cm × 1 mm length film thickness, which increases the pre-concentration efficiency (Prieto et al. 2010). The PDMS coating on the stir bar acts as an immobilized liquid into which apolar analytes in an aqueous matrix can partition. The polar matrix components (including inorganic salts, carbohydrates, ionized acids and amines) do not partition well into the PDMS because of the apolar nature of the PDMS (Baltussen et al. 1999) that significantly aids it performance in extracting VOC as sample component interferences are greatly reduced. After sampling, the extracted analytes are recovered by thermal or liquid desorption and transferred respectively to a GC-MS system for analysis. Hoffmann and Heiden (2000) identified different VOCs in milk, condensed milk, cream cheese and yoghurt samples by SBSE coated with PDMS for 60 min at 30°C. The main VOCs detected were ketones, long-chain FFAs (C:10-C:16), lactones and sulphur compounds. Schiano et al. (2019) compared SBSE (PDMS), HS-SPME (DVB/CAR/PDMS) and solvent-assisted SBSE (SA-SBSE) (PDMS) to extract vitamin degradation VOCs from fluid skim milk. The extraction conditions involved submersing the stir bar in cyclohexane for 30 min at room temperature, drying then adding to milk at 25°C for 60 min. The results showed that SA-SBSE outperformed both SBSE and HS-SPME in terms of linearity, relative standard deviation and LOD and LOQ. High et al. (2019) compared SBSE, to SAFE, HS-SPME and HS sorptive extraction (HSSE) on reconstituted spray-dried sheep milk. The authors prepared sheep's milk powder in deionized water to 20% solids (w/w). The sample preparation for SBSE (PDMS) involved immersion at 35°C for 90 min and similar conditions for HSSE analysis. For HS-SPME the reconstituted sheep milk was extracted for 60 min at 35°C SPME using the 50/30 µm DVB/CAR/PDMS fibre. For SAFE analysis 250 g reconstituted sheep milk was mixed with 100 mL of dichloromethane and distilled in the SAFE apparatus over a period of approximately 3.5 h. The organic layer was collected and dried with Na_2SO_4 (anhydrous) at room temperature under a stream of nitrogen at 100 mL/min. The authors found that SBSE was the most effective technique, with good selectivity, sensitivity and reproducibility from small sample volumes, although as anticipated some VOC selectivity exists for each technique. Typically extraction times for SBSE are longer than HS-SPME due to the enhanced phase volume, as additional time is required to enable the VOC interact with the phase.

High capacity sorptive extraction

A new high capacity passive SE technique called HiSorb (Markes International Ltd, Bridgend, UK) has been developed that is somewhat similar to SBSE, but more automatable and can also be performed as a headspace (HS) or as a direct immersion (DI) technique (Lancas et al. 2009). Cheng et al. (2021) compared DI-HiSorb (PDMS), HS-HiSorb (PDMS), TD (Tenax/Carbograph) and HS-SPME (DVB/CAR/PDMS) for the extraction of VOCs from WMP, which was reconstituted at 10% solids in ultra-pure deionized water overnight at 4°C prior to evaluation. These authors found DI-HiSorb using a non-polar GC column identified more aldehydes, ketones, lactones, esters and terpenes than HS-SPME at 40°C for 120 min. These authors also found that DI-HiSorb was particularly effective in extracting lactones in comparison to all the other extraction techniques. Faulkner et al. (2018) compared the efficiency of HS-SPME (DVB/CAR/PDMS) and DI-HiSorb (PDMS) for the extraction of VOCs in pasteurized milk samples. These authors found that an extraction/equilibration time of 60 min at 37°C by DI-HiSorb method achieved good results for pasteurized milk samples and identified 38 VOC from a range of different chemical classes, slightly more than the 36 VOC extracted by HS-SPME. Some lactones (γ-crotonolactone, σ-valerolactone, σ-decalactone, σ-dodecalactone) and p-cresol were only identified using DI-HiSorb. Clarke et al. (2022) also used DI-HiSorb (PDMS) at 40°C for 1 h to extract volatiles in raw milk and managed to successfully identify 99 VOCs consisting of acids (20), alcohols (17), aldehydes (16), esters & ethers (9), furans (3), hydrocarbons & benzenes (7), ketones (10), lactones (5), pyrazines & pyridines (4), sulphur VOC (3) and others (5).

Solvent-assisted flavour evaporation

SAFE is an extraction technique which allows the separation and concentration of volatiles by vacuum distillation. SAFE has been shown to extract a great number of aroma compounds from different chemical classes in food (Huang et al. 2019; Zhou et al. 2019). The SAFE distillation system consists of a vacuum pump and usually two cooling traps of liquid nitrogen. The sample is mixed with a solvent (usually diethyl ether or dichloromethane), and the VOCs are collected by distillation with the solvent in the first trap, while impurities and the water condense in the second trap. Engel et al. (1999) provided an overview of the procedure, where they undertook distillation for 36–240 min under vacuum (104–106 Pa) at 40–70°C using a circulation water bath. After distillation, the sample was concentrated under a stream of nitrogen and transferred to a screw-top glass tube for phase separation. SAFE enables the extraction of VOC without extensive preparation; however, it is time consuming and expensive due to the requirements for specialist glassware. It is often frequently associated with GC-O

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analysis, due to the preservation of the heat labile volatiles and lack of artifacts created through extraction at low temperature (Whetstine et al. 2006; Evans et al. 2010; Sonmezdag 2019). However, distillation-extraction techniques are becoming less favourable due to the volumes of solvents required, the time required and the variable recovery rate of highly volatile compounds (Jeleń et al. 2012).

Ning et al. (2011) compared SAFE, SDE and HS-SPME (75 µm CAR/PDMS, 65 µm PDMS/DVB and 50/30 µm DVB/CAR/PDMS) to detect VOC of fermented camel milk. A total of 26 aroma-active VOC were detected by GC-O by SAFE with dichloromethane (20 mL) at 60°C for 30 min. Compared with other pre-treatment methods, the results from SAFE proved to be effective for less volatile and more polar components (mainly alcohols and esters), but also extracted many low boiling points components such as acetaldehyde, ethanol and ethyl acetate. Smith et al. (2016) characterized the VOC profile of milk protein concentrates (MPC 70, 80, 85), milk protein isolates (MPI), acid casein, rennet casein and micellar casein concentrate (MCC) by SAFE and HS-SPME. The caseins, MPC/MPI and MCC powders were reconstituted to 10% (wt/vol) in a sodium chloride solution, and extraction was performed for 30 min at 40°C by HS-SPME with DVB/CAR/PDMS fibre. The 30 mL reconstituted powder sample was mixed with 100 mL of diethyl ether and SAFE extraction carried out for 40 min at 50°C. The extracts were concentrated under a stream of nitrogen to 20 mL. The VOCs were extracted by HS-SPME and by SAFE. SAFE detected 24 VOC not detected by HS-SPME, and HS-SPME detected 30 compounds not detected by solvent extraction (SAFE). These results highlighted that SAFE tends to favour the extraction of higher molecular weight VOCs. Evans et al. (2009) also used HS-SPME (DVB/CAR/PDMS) and SAFE to extract VOCs in milk serum protein concentrates and in whey protein concentrates (reconstituted at 10% solids, with 10% NaCl). These results demonstrated that SAFE (with 15 ml ethyl ether solvent) recovered different classes of VOCs compared with HS-SPME (DVB/CAR/PDMS) at 40°C for 25 min. Mahajan et al. (2004) investigated aroma compounds in sweet whey powder. One killogram of sweet whey powder was isolated by solvent (500 mL of 2:1 freshly distilled pentane and diethyl ether solution) extraction followed by SAFE. The most aroma-intense compounds detected by SAFE were short-chain fatty acids, aldehydes and ketones, lactones, sulphur compounds, phenols, indoles, pyrazines, furans and pyrroles. As mentioned previously, High et al. (2019) compared SAFE to HS-SPME (DVB/CAR/PDMS), HSSE (PDMS) and SBSE (PDMS) for the extraction of VOCs in spray-dried sheep milk. These authors found that SAFE was the only extraction technique capable of extracting high concentrations of both the small polar sulphur compounds (dimethyl sulfone) and also larger less volatile lactones. The diethyl ether and dichloromethane solvent were investigated in their preliminary experiment, and only dichloromethane was selected for the sheep milk SAFE extraction. This study confirmed that SAFE is suitable to extract highly polar and higher molecular weight VOCs, but is dependent upon the solvent employed. However, the authors found that SAFE was the least reproducible and the least efficient of the methods evaluated. Miyaji et al. (2021) employed SAFE (100 g yoghurt samples with 100 mL dichloromethane stirred at room temperature for 1 h) to investigate off-flavours from pasteurized drinking yoghurt made from skim milk during long-term ambient storage. Seventy eight VOC were identified. The results demonstrated that SAFE is very useful in extracting highly volatile compounds which are representative of yoghurt. Lozano et al. (2007) compared DHA (10 g butter were purging of the headspace volatiles onto a Tenax TA adsorbent tube by nitrogen at 40°C for 25 min) to SAFE (112 g butter combined with 440 mL diethyl ether at 30°C for 30 min) to analyse aroma compounds in commercial sweet cream butter by GC-O. A total of 32 and 27 aroma-active compounds were identified by SAFE and DHA, respectively. Some highly volatile compounds such as dimethyl sulphide were lost during workup and concentration using SAFE; however, less volatile compounds such as lactones were better recovered by SAFE than DHA. Sarhir et al. (2021) investigated the VOC profile of Moroccan fermented-salted 'Smen' butter and compared purge-and-trap extraction (PTE) at 36°C for 15 min with Lichrolut EN (200 mg) sorbent to SAFE (30 g butter sample with 80 mL of diethyl ether solvent) at 40°C for 30 min. A total of 27 and 30 aroma compounds were identified by the PTE and SAFE, respectively, but significant differences in the VOCs extracted existed between both methods. The results demonstrated that SAFE was more efficient in the extraction of carboxylic acids than PTE, and the aroma-active compounds detected using SAFE had higher flavour dilution (FD) factors demonstrating that greater concentrations were extracted.

Dynamic extraction (DE)

In dynamic methods, such as purge and trap (P&T) and TD, the dairy sample is typically heated and the VOCs continuously removed and subsequently concentrated in a cold trap, or adsorbed onto an inert support prior to injection onto the GC. Valero et al. (1997) described the general process used in TD, where VOC are trapped into TD tubes using an inert gas such as nitrogen or helium. In their study, tubes were subsequently desorbed to cold trap to aid peak focussing prior to desorption to the GC. A wide range of absorbent and adsorbent trapping materials are available and flows can be controlled to split extracts that gives a lot of possibilities to enrich or dilute extracts with relative ease. Samples amounts can be relatively large as the loading capacity of the tubes are large which is beneficial for trace analyte

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detection (Valero et al. 1997). Cheng et al. (2021) evaluated WMP using TD and had additional equipment such as a Micro-Chamber/Thermal Extractor (μ-CTE) (Markes International Ltd) that provided greater control in the process. These authors used a Tenax/Carbograph sorbent and found that TD was more effective at extracting aldehydes, ketones, alcohols and benzene/phenols, but ineffective for lactones. However, some VOC that were not extracted by DI-HiSorb, HS-HiSorb, or HS-SPME but were detected by TD (longifolene, α-terpineol, 1-nananol, p-xylene and 2,3-pentanedione).

P&T is a good technique for the detection of highly volatile compounds with lower boiling points, such as alcoholic compounds and is solvent-free (Mallia et al. 2005), but has generally been surpassed by more automatic extraction methods. Using the P&T technique, the dairy sample is usually homogenized with water, placed in a U-shaped glass sparger and heated. Subsequently, an inert gas (nitrogen or helium) is purged through the sample to transfer the VOCs to an inert support of trapping material, which is thermally desorbed and concentrated once again in a cold trap (cryofocusing) before injection onto the GC-column. A wide range of trapping materials are available. Contarini and Povolo (2002) compared to P&T (at room temperature for 60 min with a Tenax trap) and HS-SPME (at 45°C for 30 min with DVB/CAR/PDMS fibre). Both P&T and HS-SPME were comparable in terms of repeatability. The results demonstrated that 11 VOCs were obtained from the milk samples by both PT and HS-SPME. The P&T technique was also better able to extract smaller molecular weight VOCs (such as, acetone and 2-butanone). Naudé et al. (2009) developed a novel P&T sampling method to extract VOCs from long life UHT milk (2% milk fat) by trapping it on a multi-channel open tubular traps of PDMS for at 45°C for 35 min in nitrogen at 25 mL min^{-1}. The VOCs were subsequently desorbed from the cold traps to the GC using a TD-type system. The authors found that dimethyl sulphide, 2-methylpropanal, 2,3-butanedione, 3-methylbutanal, 2-hexanone, 2-heptanone, 2-nonanone, nonanal and decanal were the predominant VOCs in these samples. Francesca et al. (2015) exploited the potential applications and setup conditions of the automated Gerstel TD (Gerstel GmbH & Co, M lheim, Germany) using microporus sinthered glass (TDU-CIS4–GC–MS) and cryogenic trapping for the identification of oxidized or non-oxidized volatile compounds of powdered milk at 30°C for 30 min. They identified 17 VOC mainly consisting of aldehydes, ketones, acids and alcohols. Ciccioli et al. (2004) developed a multiple dynamic headspace extraction TD system for the accurate determination of VOC in goat milk samples. The authors used a series of different traps in an attempt to obtain as true a volatile profile as possible (Tenax and different types of Carbograph) where helium was passed through the sample at 200 mL min^{-1} at 50°C to dynamically extract the VOC onto the tubes. The authors also used a colder empty trap before the packed traps in an attempt to reduce moisture getting onto the packed traps. These authors identified 33 VOC mainly consisting of aldehydes, ketones, terpenes, alcohols and benzene compounds in goat's milk.

VOLATILES - MILK, DAIRY POWDERS, BUTTER AND YOGHURT

Key volatiles associated with the aroma of milk and dairy powders

VOC including aldehydes, ketones, alcohols, lactones, phenols and esters in milk products originate from the degradation of the major milk constituents (lactose, citrate, milk lipids and milk proteins) (Cadwallader and Singh 2009), but many are also created through rumen metabolism can also be directly transferred from diet (Kilcawley et al. 2018; Clarke et al. 2022).

Typically, the most abundant VOC chemical class in many dairy products are short-chain carboxylic acids, known to be major components responsible for the sour taste (Coppa et al. 2011; Villeneuve et al. 2013) and in some cases rancidity (Kilcawley et al. 2018). There are derived from various sources and pathways; lipolysis, carbohydrate metabolism or amino acid metabolism depending upon the specific carboxylic acid (Kilcawley et al. 2018). Simple acids (<6 carbon) have high odour thresholds, while long chain acids (12 or more carbons) are odourless. Unsaturated acids generally have sharper and stronger odours than saturated ones (Jeleń et al. 2012). A recent study by Clarke et al. (2022) found that butanoic acid (cheesy, dairy, buttery) was a major contributor to the aroma of raw cow's milk. Karagül-Yüceer et al. (2001) determined that butanoic (cheesy), hexanoic acids (cheesy), octanoic acid (waxy, soapy) and dodecanoic acids (fat, sweet) were detected at high odour intensities in the acidic fraction of nonfat dry milk. Karagül-Yüceer et al. (2002) also found that octanoic, nonanoic, decanoic and dodecanoic acids were associated with soapy/waxy/rubbery attributes in stored nonfat dry milk. These authors also found that octanoic and decanoic acids had very high FD factors and that sour taste was correlated with pentanoic acid. Moreover, propionic acid, 2-methylpropionic acid, 2-/3-methylbutanoic and pentanoic acids with sweaty or Swiss cheese-like aroma notes were present in the acidic fractions of these nonfat dry milks.

Primary aldehydes are mainly derived from oxidation of polyunsaturated fatty acids (PUFA), but can also be transferred from plant material into milk (Kilcawley et al. 2018; Clarke et al. 2022). The impact of oxidation on VOC generation in milk and in many dairy products is significant, as the fatty acid profile of milk, especially PUFA is greatly impacted by diet (O'Callaghan et al. 2019). The chain

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length of aldehydes mostly affects odour thresholds and odour properties. Aldehydes with low molecular weights (<150 Da) tend to be associated with unpleasant odours, and those with higher molecular weights tend to have sweet, fruity odours (Giri et al. 2010). Milk produced from the cows fed pasture (perennial ryegrass or perennial ryegrass and white clover) was higher in linolenic acid content, which is known to influence the degree of lipid oxidation (O'Callaghan et al. 2016). Havemose et al. (2006) found the level of other primary aldehydes such as hexanal, heptanal and pentanal increased in milk produced from cows fed grass/clover silage after exposure to fluorescent light compared to milk produced from a hay diet. Feeding pasture has also been shown to significantly elevate the levels of 2-nonenal, hexanal and octanal in milk (Glover et al. 2012). Pentanal is a product of the autoxidation of arachidonic and linoleic acid and was found at greater intensities in milk from cows fed pasture and silage than in milk from cows fed just hay (Villeneuve et al. 2013; Clarke et al. 2020a). Pentanal has also been associated with the cardboard-like or metallic-like off-flavours in milk after prolonged exposure to light (Zardin et al. 2016). Francesca et al. (2015) associated pentanal, hexanal, octanal, 2-heptenal, nonanal, 2-octenal, 2-nonenal, 2-decenal, 2,4-nonadienal, 2-undecenal, 2,4-decadienal with oxidation in powdered milk, defined as 'pungent', 'green (or herbaceous)', 'fat' and 'food-fried'. Boltar et al. (2015) noted that the primary aldehydes nonanal and octanal (products of lipid-oxidation) were significantly higher in milk produced from winter grass silage also highlighting an impact of diet on lipid oxidation. Coppa et al. (2011) found higher benzeneacetaldehyde concentrations in milk from cows on rotational grazing than in milk from a hay-based diet, or from cows on continuous grazing. Benzeneacetaldehyde is primarily derived from phenylalanine metabolism, but may also be transferred directly into the diet (Coppa et al. 2011; Kilcawley et al. 2018; Clarke et al. 2021). The Strecker aldehydes 2- and 3-methylbutanal (grassy, fatty, astringency and painty) were found to be more abundant in WMP produced from milk of cows fed hay than cows fed diets of maize silage or grass silage and results from the metabolism of isoleucine and leucine, but are also involved in the Maillard reaction (Lloyd et al. 2009).

Ketones are also mainly derived from oxidation of FA in dairy products, but some are also the result of carbohydrate metabolism, it has been suggested that many may not have a significant impact on milk flavour due to their relatively higher odour thresholds and relatively low concentration (Kilcawley et al. 2018). In heat-treated milk, ketones are mainly products of the heat-initiated decarboxylation of β-oxidized saturated fatty acids or decarboxylation of β-ketoacids (Jansson et al. 2014). Contarini et al. (1997) noted that ketones having a higher carbon number are responsible for heated milk flavour. These authors demonstrated that the abundance of 2-heptanone and 2-pentanone increased in milks stored at room temperature and were responsible for heated milk flavour. Moreover, acetone and 2-butanone were also lower in UHT milk and are thought to derive mainly directly from cow's diet (Contarini et al. 1997). Coppa et al. (2011) found that 2,3-octanedione was more abundant in milk derived from diverse pastures and suggested this was due to oxidation of linoleic acid and linolenic acid. Clarke et al. (2020b) found that 3-octen-2-one was correlated with 'caramelised flavour' and 'sweet taste' in WMP. Vazquez-Landaverde et al. (2005) noted that 2-pentanone, 2-hexanone, 2-heptanone, 2-nonanone and 2-undecanone have been identified as thermally derived off-flavours linked to the level of fat in the milk. Clarke et al. (2020b) found that 3,5-(E,E)-octadien-2-one (grassy, fruity and green), a product of linolenic acid oxidation, was significantly higher in pasteurised milk derived from concentrate and correlated with hay-like flavour. Clarke et al. (2022) found that 2,3-butanedione, a product of pyruvate metabolism (fresh, sweet, caramel, butterscotch, biscuit and baked), was a key odourant of milk from cows outdoors on pasture (perennial ryegrass).

Sulphur volatiles are potentially very important aroma compounds due to their high odour activities (Falchero et al. 2010). Kobayashi et al. (2008) found methyl 2-methyl-3-furyl disulphide, furfuryl methyl disulphide and bis(2-methyl-3-furyl) disulphide were present in high heat-treated SMP and in UHT milk, which presented a 'canned corn-like', 'rice bran-like' and 'vitamin-like' odour profile. Vazquez-Landaverde et al. (2005) found that dimethyl sulphide was almost three times higher in UHT than in raw milk and was formed from the sulfhydryl group of milk proteins subjected to thermal denaturation. Clarke et al. (2022) found that methanethiol (cabbage) was an important odorant in raw cow's milk from pasture (perennial ryegrass).

Terpenes are naturally occurring plant secondary metabolites derived from isoprene units (C5) and also derived from larger terpenoids; monoterpenes (C10) and sesquiterpenes (C15). Terpenes are odour active but have a high odour threshold and therefore need to be at high concentrations to have a sensory impact (Kalač 2011). Ciccioli et al. (2004) noted the maximum monoterpene (α- and β-pinenes) content in milk was associated when the largest variety of herbs was present in the pasture. Faulkner et al. (2018) also found that β-pinene is most likely derived directly from forage, but concentrations are dependent upon the diversity of the pasture. These authors also found that β-pinene was absent in cow's milk derived from a concentrate diet. Coppa et al. (2011) found the concentrations of β-pinene and cymene-(p) and all sesquiterpenes (β-caryophyllene, alloaromadendrene, germacrene-D and γ-cadinene) were higher in milk derived from animals on continuous grazing than on less diversified pasture under rotational grazing. Coppa et al. (2011) also found that sesquiterpenes were more

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influenced by different grazing systems than monoterpenes. Limonene (sweet citrus-like) is also a product of bioconversion of sesquiterpenes and was the most common terpenes in milk from a range of highland and lowland pastures (ryegrass, clover) or from concentrates (maize silage, hay and cereals) over different seasons (Fernandez et al. 2003).

Phenolic compounds can be important volatile compounds in milk related to forage intake. Alkylphenols in ruminant milks are derived from phenolic compounds ingested through feed and were responsible for the 'cowy flavour' of milk (Feo et al. 2006). p-Cresol is a major alkylphenol and has a characteristic 'barn-like flavour' that blends with the more medicinal notes of m-cresol in milk (Ha and Lindsay 1991). Faulkner et al. (2018) found a direct link between p-cresol levels in raw milk from cows fed clover with 'barnyard aroma', which was also subsequently linked to isoflavone metabolism by Clarke et al. (2019). Karagül-Yüceer et al. (2002) noted that p-cresol and skatole may be the contributors to undesirable flavours in milk. Phenols (clove-like, medicinal and smoky) are described as heat-generated compounds in UHT milk (Dursun et al. 2017). Most phenolic compounds are excreted, but some end up in milk and depending upon their abundance may influence sensory perception.

Hydrocarbons compounds with high odour thresholds can also play an essential role in food aroma when present at high concentrations (Czerny et al. 2011). Toluene, a product of β-carotene light-induced oxidation, has been implicated as responsible for rancid notes and was more abundant in pasture-derived milk than milk from cows fed indoors (Coppa et al. 2011). Faulkner et al. (2018) found toluene was significantly higher in CLV milk than concentrate milk and linked as a potential biomarker for pasture, derived from metabolism in the rumen. Xylene (sweet) may be the result of carotenoid degradation, namely β-carotene degradation in the rumen or possibly directly transferred from feed (Buchin et al. 1998).

Lactones are cyclic compounds formed by the intramolecular esterification of hydroxyacids through the loss of water, described as having a buttery-type, creamy, fruity or otherwise pleasant odour. Few differences in lactone content were linked to diet, but they appear to be more important in pasteurized milk than in raw milk because heat is a factor in their production (Urbach 1997; Li et al. 2020). Villeneuve et al. (2013) found the detection intensity of δ-octalactone and δ-tetradecalactone were affected by forage types. In their study, the content of γ-decalactone, γ-dodecalactone and γ-dodecaenolactone in milk was higher in hay-fed cows, lower in silage-fed cows and intermediate for cows on pasture. Karagül-Yüceer et al. (2002) showed that δ-decalactone and γ-dodecalactone gave sweet odour properties to milk powder. Sweet and milky odour properties were characterized by lactones including γ-undecalactone, γ-dodecalactone, δ-decalactone and δ-undecalactone. Clarke et al. (2022) found that γ-butyrolactone was an important odour active volatile in raw cow's milk, and that γ-hexalactone influenced the aroma of cow's milk produced from a concentrate diet.

Key volatiles associated with the aroma of butter

Garvey et al. (2020) found pentanal (paint-like) and decanal (green, fatty), derived from oleic acid and linoleic acid (also arachidonic acid for pentanal), were more abundant in butter produced from cows outdoors fed perennial ryegrass and white clover than perennial ryegrass alone or from cows indoors fed concentrate. In their study, heptanal was significantly more abundant in butters produced from milk derived from a pasture (perennial ryegrass or perennial ryegrass and white clover) in comparison to a concentrate diet and has a 'green sweet' aroma. Glover et al. (2012) noted butanoic acid levels were higher in butter produced from milk from cows fed the concentrate compared with pasture. Butanoic acid is likely a very important aroma compound in butter and was a main contributor to 'fresh butter' aroma in sweet cream butter (Lozano et al. 2007). O'Callaghan et al. (2016) found that acetone (earthy, strong fruity and hay) was significantly correlated with butter produced from milk derived from cows outdoors on perennial ryegrass and white clover diets, than in butter produced from milk from cows outdoors on perennial ryegrass, or indoors on concentrate. These same authors also found 2-butanone (buttery, sour milk and etheric) was significantly more abundant in butter produced from concentrate diets. Mallia et al. (2008) found that the concentrations of 1-octen-3-one (mushroom) increased when butter oil was stored at room temperature. Li et al. (2020) found 3-penten-2-one is a product of lipid oxidation, with low levels indicating freshness in butter. 2,3-Butanedione is a very odour-active compound with a characteristic buttery aroma, derived from pyruvate (Liu et al. 2020b). Garvey et al. (2020) found 2,3-butanedione was significantly more abundant in butter produced from milk derived from cows outdoors fed perennial ryegrass and white clover compared to butter produced from cows indoors on a concentrate diet. Li et al. (2020) found that δ-decalactone was the most important odour active aroma in butter, and that overall lactones in general were important odour compounds in butter. Lozano et al. (2007) compared the VOC profile of fresh sweet cream butter and butters stored at refrigeration (4°C), frozen (−20°C) and at room temperature. These authors identified butanoic acid, δ-octalactone, δ-decalactone, 1-octen-3-one, 2-acetyl-1-pyrroline, dimethyl trisulphide and 2,3-butanedione as the most intense aroma compounds associated with fresh butter samples and that dimethyl sulphide is possibly a contributor to cooked/nutty flavour in butter, which is in agreement with Peterson and Reineccius (2003. Lozano et al. (2007) also noted that the main changes in aroma active VOC over storage were related to an increase in the intensity of lactones (δ-octalactone, δ-decalactone and

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δ-dodecalactone), lipid oxidation VOC ((E)-2-nonenal, 2-heptanone, (Z)-4-heptenal, (E,Z)-2,6-nonadienal and hexanal) and acidic odourants such as acetic and butanoic acids. These authors also noted that styrene levels increased over storage due to migration from packaging material and may adversely impact on fresh butter flavour.

Lozano et al. (2007) also suggested that toluene (nutty, bitter, almond and plastic) may be associated with stale butter flavour it is a product of β-carotene degradation and has been previously shown to be significantly higher in butter derived from milk of cows fed outdoors on pasture (perennial ryegrass or perennial ryegrass and white clover) than cows indoors fed concentrate (O'Callaghan et al. 2016).

Key volatiles associated with the aroma of yoghurt

Acetaldehyde, predominantly derived from pyruvate decarboxylation or generated by the metabolism of threonine, is a major aroma compound in yoghurt and exhibits a green apple or nutty flavour (Cheng 2010; Eram and Ma 2013; Settachaimongkon et al. 2014). Tian et al. (2017) demonstrated that the concentration of acetaldehyde increased after the end of fermentation, reached a maximum at the beginning of storage and then declined sharply with increasing storage time. This study highlighted that yoghurt samples fermented with a *Lactobacillus acidophilus* culture produced the highest concentrations of acetaldehyde in comparison to other strains evaluated. Tian et al. (2019) also demonstrated that acetaldehyde contributes a 'green apple' or 'nutty' attribute at lower concentrations, but negatively influences aroma at high concentrations. 2,3-Butanedione and acetoin are produced by pyruvate or citrate metabolism by various lactic acid bacteria and are typical carbonyl compounds and contribute greatly to the 'butter and cream' aroma of yoghurt (Hugenholtz 1993; Neves et al. 2005). Acetoin derives from the enzymatic degradation of 2,3-butanedione and although has a much weaker aroma than 2,3-butanedione helps to contribute to a 'mild creamy' aroma in yoghurt (Cheng et al. 2010). Innocente et al. (2016) found that a 1:1 acetaldehyde to 2,3-butanedione ratio gave the most preferential yoghurt aroma, while too much acetaldehyde resulted in a 'green off-flavour'. A study by Tian et al. (2017) demonstrated that 2,3-butanedione and acetoin reached maximum concentrations after 14 days refrigerated storage post production. Acetoin, 2,3-butanedione and 3-heptanone are also all known to contribute to yoghurt odour by providing 'fruity, sweet' aromas (McSweeney and Sousa 2000; Gallardo-Escamilla et al. 2005). Tian et al. (2017) reported that 2-butanone, 2-pentanone and 2-heptanone (originating from oxidation, carbohydrate metabolism and/or direct transfer) were all identified as significant aromatic volatiles in yoghurt samples fermented with *Lactobacillus casei*. Short-chain fatty acids are also produced during yoghurt fermentation by both lipolytic processes and by lactic acid starter fermentation (Tamine and Richard 2007). Acetic acid, one of the important acidic compounds produced by hetero fermentative LAB, contributes an undesirable vinegar taste at high concentrations, which can unbalance the overall flavour (Buffa et al. 2004). However, as acetic acid is not that odour active, excessive levels are generally not a major issue in yoghurt production. Innocente et al. (2016) demonstrated that both hexanoic and butanoic acids were significantly higher in yoghurt samples fermented with *Lactobacillus casei* than yoghurt fermented with *Lactobacillus rhamnosus*. Tian et al. (2019) documented that butanoic and octanoic acids contribute to the characteristic cheese flavour of yoghurt, and that decanoic acid provides a 'light cream' flavour. Some alcohol compounds also contribute to the aroma of yoghurt. Lower alcohols (from C1 to C10) affect the flavour of yoghurt and can be important as they positively influence sensory perception (Cheng 2010). Ethanol, the final product of glucose metabolism or amino acid degradation in milk, is thought to influence sweetness (Urbach 1995), but unlikely to have a major contribution due to its very high odour threshold. Tian et al. (2019) demonstrated that 3-pentanol and 1-hexanol contribute to a 'grass' flavour in yoghurt. 1-Pentanol and 2,3-butanediol were demonstrated to provide a 'fruit' flavour and improve the overall flavour quality (Tian et al. 2019).

GAS CHROMATOGRAPHY OLFACTOMETRY – MILK, DAIRY POWDERS, BUTTER AND YOGHURT

In GC-O, the human nose is used as a detector to evaluate the character and odour intensity of VOCs (Zellner et al. 2008). Thus, it is possible to discern key aromatic compounds in dairy products by GC-O, but impossible to completely understand the whole aroma profile using GC-O, partly because other factors influence aroma perception, but also because aromas often consist of a combination of two or more VOC. As previously stated, VOCs are challenging to extract, separate, identify and quantify as they can interact synergistically or additively to produce an overall odour (Brattoli et al. 2013). Even though GC-O has existed for decades, it remains a relatively obscure research technique especially for milk, dairy powders, butter or yoghurt (~10 publications to date). Overall, the limited use of olfactory analysis for these products is difficult to fathom as even though it is not a complete solution in relation to fully understanding the relationship between VOC and aroma perception, it does provide a very good insight into the aroma characteristics of a product. A likely aspect for its limited use is that it requires highly trained assessors and is quite time consuming (Zellner et al. 2008). However, the potential benefits easily outweigh any disadvantages. GC-O and chemical sensor technologies such as electronic nose and tongue (e-nose and e-tongue), combined with multivariate

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data processing methods, are promising relatively novel approaches for rapid analysis of food (Wardencki et al. 2013). Merging both GC-O and GC–MS as an integrated instrument is particularly useful for the identification of aroma-active VOC.

To date, HS-SPME, SAFE and dynamic headspace sampling (DHS) are the most commonly used as pre-treatment methods for GCO analysis (Song and Liu 2018). VOC are typically present from trace amounts to even a few mg kg^{-1} (such as fatty acids in cheese) with odour thresholds varying from ppt to many ppm. In strong smelling dairy products, it may not be necessary to concentrate the VOC profile for GC-O, but for products such as fresh milk it is necessary, thus the choice of extraction method can be dependent upon the sample type. However, care must be taken in GC-O to avoid losing thermal labile VOC or specifically enhancing or creating VOC during the extraction processes, otherwise spurious information may be generated.

Table 3 highlights the odour active VOCs associated with milk, milk powder, butter and yoghurt products. A recent study employed HS-SPME-GC-O (8 mL milk sample with 2 g of sodium chloride at 50°C for 40 min extraction with 75 µm DVB/CAR/PDMS), to compare the volatile profiles of raw and pasteurized milk and pulsed electric field (PEF) treated milk (Zhang et al. 2011). PEF is a nonthermal processing technology that can be applied to liquid milk to inactivate both spoilage and pathogenic microorganisms but also maintains the original nutrients of milk (Amiali and Smith 2007). In the study by Zhang et al. (2011), a total of 19 active VOC were detected with aldehydes making major contributions to a 'fruity, green, cream' note in both pasteurized and PEF-treated milk. 2 (5H)-Furanone was only detected in PEF-treated milk and described as 'caramel' odour. Although concentrations of aldehydes and methyl ketones differed between pasteurized and PEF-treated milk, it appeared not to impact their aroma activities. Colahan-Sederstrom and Peterson (2005) determined if epicatechin addition to raw milk would inhibit the thermal generation of Maillard-type aroma compounds in UHT-processed fluid milk. A total of 32 aroma-active VOC were identified in UHT milk using SAFE-GC-O and GC-MS (1 kg milk sample was extracted with 875 mL diethyl ether for 1 h at 40°C). Methional, furfural, 2-isopropyl-3-methoxypyrazine, 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline (Maillard-type aroma compounds) showed the largest changes in FD post heat treatment and contributed to the 'cooked' and 'bitterness' flavour of UHT milk. This study demonstrated that epicatechin had the greatest inhibitory effect on the Maillard-derived compounds. In another study, a direct solvent extraction and high-vacuum distillation extraction method was developed for detection of chemical and sensory profiles of stored nonfat dry milk by GC-O (Karagül-Yüceer et al. 2002). Fifty six aroma active VOCs were detected, and a variety of aldehydes, ketones, alcohols and free fatty acids were found to be responsible for the development of undesirable flavours. These authors stated that p-cresol, 3-methylindole (skatole) and some unknown compounds with 'cowy', 'fecal' or 'animal-like' odours appear to contribute to undesirable flavour in milk. Methional and o-aminoacetophenone had high odour intensities in these nonfat dry milks and had characteristics 'boiled potato' and 'animal' odours, respectively. Free fatty acids, including butanoic and hexanoic (cheesy notes) and octanoic, nonanoic, decanoic and dodecanoic acids (waxy note), were also found to contribute to the aroma of milk. Sun et al. (2021) investigated key aroma-active compounds in butter by SAFE-GC-O and GC-MS (40 g butter distilled with 200 mL dichloromethane at room temperature for 30 min). Fifty-three odorants were identified. 2-Furfurylthiol, 2-acetylthiazole, anethole, (E)-2-decenal and 1,8-cineole were the key odorants for the overall aroma of butter and contributed the 'beef', 'boiled beef', 'anise', 'tallow', 'mint, herb' aromas, respectively. As previously mentioned, Lozano et al. (2007) investigated the major aroma components of sweet cream butter. These authors identified 32 and 27 aroma-active VOC were identified by SAFE-GC-O and DHS-GC-O, respectively. VOCs such as lactones were easily recovered by SAFE but poorly by DHS. Butanoic acid, 1-octen-3-one, 2,3-butandione, 2-acetyl-1-pyrroline, dimethyl trisulphide, δ-octalactone and δ-decalatone were the main contributors to fresh butter aroma. Peterson and Reineccius (2003) determined key odourants in heated sweet cream butter aroma by using static headspace analysis (5.6 g butter solution placed in P&T vessel for 45 min extraction at 38°C by Tenax TA trap) by GC-O. These authors identified 19 odour-active VOC in the HS of heated butter. Methanethiol, methional, 3-methylbutanoic acid, 2-heptanone and furaneol were the key odour-active VOC in heated butter in comparison to fresh butter and contributed to the 'pungent', 'cooked potato', 'cheesy', 'blue cheese' and 'sweet caramel' aroma, respectively. Liu et al. (2022) evaluated the odour-active VOC of yoghurt using DHS, SPME, SAFE and SBSE/GC-O and by GC-MS. A total of 31 odour-active VOC were perceived by four extraction methods with DHS providing the most VOCs. 2,3-Butanedione, hexanoic acid, acetophenone, 2,3-pentanedione, acetic acid, octanoic acid, 3-methyl-2-buten-1-ol, butyl acrylate, 2-heptanone, ethyl 2-methylbutyrate and ethyl butyrate were identified as the key odour-active components of yoghurt by DHDA (dynamic headspace dilution analysis). Aroma extraction dilution analysis and OAV identified 'green apple-like', 'sweat-like', 'sweet-like', 'fruit-like', 'butter-like', 'vinegar-like', 'red bean-like', 'green-like' and 'cream-like' flavour properties in these yoghurts. 2,3-Butanedione was found to be the most important odour-active VOC with the highest FD value in yoghurt, contributing 'buttery' odour.

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Table 3 Odour active volatile compounds identified in milk, milk powder, yogurt and butter by GCO-MS

Compounds	Odour description	References			
		Milk	Milk Powder	Yogurt	Butter
Alcohols					
Methanethiol	Pungent, sulphury				f
Ethanol	Floral, medicine				g
3-Methyl-1-butanol	Floral-fresh, cheesy, rubber, painty	c			g
2-Heptanol	Fatty-oily				g
2,3-Butanediol	Creamy			e	g
1,3-Butanediol	Musty-wet				g
1-Octen-3-ol	Mushroom, earthy	a, b			h
1-Heptanol	Mushroom				h
Furfuryl alcohol	Caramel				h
α-Terpineol	Green				h
2-Butanol	Sweet almond-like			e	
1-Butanol	Balsam-like, burnt, sweet	c		e	
1-Pentanol	Sweet			e	
1-Hexanol	Greasy			e	
2-Ethyl hexanol	Citrus			e	
2-Phenyl ethanol	Rose		j		
Aldehydes					
2-Methyl propanal	Dark chocolate				i
2-Methyl butanal	Dark chocolate				i
3-Methyl butanal	Dark chocolate, sweet, fruity, fatty		j	e	i
Hexanal	Green, grass, tallow, fruity, floral	b, c, d	j	e	h, i
(Z)-4-Heptenal	Rancid, crabby, biscuit-like	b	j		i
Nonanal	Mushroom, waxy, fatty, floral, green, rosy, sweet, floral	d		e	h, i
Decenal	Green, fatty, floral			e	h, i
(E)-2-Nonenal	Hay, green, fatty, cucumber, oxidized	a, b	j		f, i
(E,Z)-2,6-Nonadienal	Cucumber, cardboard	a, b	j		i
Acetaldehyde	Green, pungent, apple like			e	f
Benzaldehyde	Almond-nutty			e	g
Heptanal	Fat, citrus, cheesy, caramel, fruity	c, d			h
(E)-2-Hexenal	Apple, green				h
Octanal	Fat, soap, orange, fragrant, citrus	c, d		e	h
(E)-2-Heptenal	Fat, fruity	c			h
(E,E)-2,4-Hexadienal	Fat, green	c			h
(E)-2-Octenal	Green, fatty	b, c	j		h
(E,E)-2,4-Heptadienal	Fat, green	c			h
(E)-2-Decenal	Tallow				h
(E)-2-Undecenal	Fat, metallic	b			h
(E,E)-2,4-Decadienal	Fat, soapy, hay, fried	b	j		h
(E,E)-2,4-Nonadienal	Cardboard	a	j		
Butanal	Cocoa-like			e	
Pentanal	Fermented like, fruity, floral	d		e	
Methional	Cooked potato	a, b			f
Phenylacetaldehyde	Rose		j		
Carbonyl compounds					
Ethenylbenzene (styrene)	Styrene, plastic, overripe fruit, clean		e	g	i
p-Cresol	Cowy, barny	b	j		
Toluene	Painty	c			
Benzothiazole	Rubber		j		

(continued)

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Table 3 (Continued).

Compounds	Odour description	References			
		Milk	Milk Powder	Yogurt	Butter
Ketones					
2,3-Butanedione (diacetyl)	Buttery, cream, cheese	b, d	j	e	f, i
1-Hexen-3-one	Plastic, veggie, rubbery	b	j		f, i
2-Heptanone	fatty, blue cheese, cheesy-nutty, sweet, fruity, milky, plastic	d		e	f, g, i
Acetoin	Buttery-creamy, mild creamy			e	g
2-Nonanone	Milky, sweet, herb-like			e	g
Acetone	Fruity			e	
2-Butanone	Fruity, buttery, cheese	d		e	
2-Pentanone	Wine-like, malty, fruity	d		e	
3-Hexanone	Rum-like			e	
2,3-Pentanedione	Sweet			e	
3-Heptanone	Green			e	
2-Undecanone	Fruity			e	
Acetophenone	Sweet-almond			e	
(Z)-1,5-Octadien-3-one	Metallic		j		
Lactones					
δ-Octalactone	Herbaceous, peach	a			f, i
γ-Nonalactone	Peachy				i
δ-Decalactone	Waxy, sweet, coconut, hot milk	a, b, d	j		h
δ-Undecalactone	Coconut, butter, green, cilantro	b	j		i
δ-Dodecalactone	Coconut, cheesy, sweet, fruity	d		e	i
γ-Dodecalactone	Sweet, green	b	j		
δ -Decanolactone	Peach				f
δ -Hexanolactone	Creamy, chocolate, sweet aromatic				f
γ-Decalactone	Sweet, perfume	a			
γ-Butyrolactone	Creamy			e	
Sulphur compounds					
Dimethyl sulphide	Sulphur, sweet			e	i
Dimethyl trisulphide	Cabbage, garlic, sulphury	b	j		f, i
Ethyl disulphide	Gasoline		j		
Hydrogen sulphide	Boiled egg, eggy				f
Dimethyl disulphide	Vegetable-like			e	
Esters					
Ethyl acetate	Fruity, mild, sweet, solvent	d		e	i
Ethyl butanoate	Fruity, berry, fruity-rose, herb-like, sweet			e	g, i
Ethyl lactate	Creamy-whey				g
Ethyl octanoate	Floral				g
Ethyl decanoate	Fruity-pear				g
Ethyl propionate	grape-like			e	
Methyl butanoate	pineapple-like			e	
Butyl propionate	Rosy, sweet			e	
Ethyl hexanoate	pineapple-like			e	
Furans					
Furaneol	Sweet caramel-like	a			f, h
Furfural	Almond, roasted, nutty	a, c			h
2-Furanmethanol	toast bread-like, vitamin, rubber, caramel	b	j	e	h
2-Acetylfuran	Plastic, nutty	c			
Acids					
Acetic acid	Vinegar, sour	c		e	h, i
Butanoic acid	Fecal, cheesy, rancid, ripened cheese, buttery, sour, creamy	a,d		e	f, g, i

(continued)

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Table 3 (Continued).

Compounds	Odour description	References			
		Milk	Milk Powder	Yogurt	Butter
3-Methylbutanoic acid	Sweaty, cheesy, whey-flowery, sour	a,b			f, g, i
Hexanoic acid	Doughy, sweaty,cheesy acrid,rancid, buttery-soapy,sour	a, b, d		e	f, g, i
Propanoic acid	Fatty, cheesy			e	g
2-Methylpropanoic acid	Rancid buttery			e	g
Pentanoic acid	Cheesy-musty, swiss cheese	b			g
Octanoic acid	Cheesy, goat, foul	a		e	g
Nonanoic acid	Green, fat, sour	a		e	g
Decanoic acid	Soapy, rot-like	a		e	
Heptanoic acid	Sour	a		e	
Dodecanoic acid	Waxy	b			
Tetradecanoic acid	Coconut-like			e	
Terpenes					
α-Pinene	Mint, pine oil, dry, woody	c			i
D-Limonene	Citrusy, Lemon, orange			e	g, h
β-Myrcene	Balsamic, rosin				h
3-Methylthiophene	Plastic		j		
β-ionone	Hay		j		
Other					
2-Acetyl-1-pyrroline	Popcorn, roasted	a, b	j		i
2-Acetyl-2-thiazoline	Cooked, popcorn, roasted	a, b	j		i
Skatole	Skatole, fecal, mothball		j		i
Acetylpyrazine	Roast				h

Abbreviation: DE, Dynamic extraction; SAFE, Solvent-assisted flavour evaporation; SBSE, Stir bar sorptive extraction; SPME, Solid-phase microextraction.

The data adapted from (a) Colahan-Sederstrom and Peterson (2005); (b) Karagül-Yüceer et al. (2002); (c) Yeh et al. (2017); (d) Zhang et al. (2011); (e) Liu et al. (2022); (f) Peterson and Reineccius (2003); (g) Sarhir et al. (2021); (h) Sun et al. (2021); (i) Lozano et al. (2007); (j) Karagül-Yüceer et al. (2002).

CONCLUSIONS

Significant advances in our understanding of the key aroma active VOC that impact the sensory perception of milk, dairy powders, butter and yoghurt have been outlined. In terms of sensory approaches, both traditional and novel sensory techniques have been discussed, but also cultural factors influencing choice. This review has reiterated the importance of product familiarity and how critical this is in relation to cross-cultural sensory acceptance, especially in countries where dairy products have little tradition. Much more sensory research of dairy products is required to better understand cultural factors influencing choice/acceptability and to ensure that all participants in such studies unambiguously comprehend what is required of them, most notably avoiding words that could be misinterpreted or have dual meanings from a cultural or language perspective.

The importance of VOCs impacting the aroma of milk, dairy powders, butter and yoghurt are discussed. More than 300 different VOC, belonging to 10 or more chemical classes, have been identified in milk, dairy powder, butter and yoghurt to date. This review has focussed on GS-MS, which is by far the most widely used approach to identify these compounds, but with particular emphasis on the different VOC extraction techniques used, highlighting their advantages and/or shortcomings. Aldehydes, alcohols, lactones, ketones, acids, terpenes, carbonyl compounds and furans are by far the most prominent and potent VOC that appear to influence the sensory appeal of these products. A single or multiple source can be responsible for the generation of VOC. Some are directly or indirectly dietary related, in that they can be transferred from the diet by ingestion or inhalation, or indirectly created during rumen metabolism and end up in the milk. Others are created during processing for example by heat treatments or by the inclusion of ingredients/processing aids or in final product formulation. Thus diet and milk quality plus product processing and formulation have a major role in VOC formation in the final product, which subsequently impacts on aroma generation and thus sensory perception.

As product variation within these dairy products is relatively large, and as a wide range of odour active VOC are

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typically present, it is difficult to absolutely identify individual VOC responsible for the overall aromatic characteristics of these dairy products. However, some informed conclusions can be made based on research to date. This review has highlighted the benefits of GC-O, especially in combination with complementary techniques such as GC-MS and also highlights that much more research is required combining sensory and analytical techniques in order to better understand flavour development in these products in order to improve quality but also adjust in-farm and process inputs to create products more suited to particular markets.

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AUTHOR CONTRIBUTIONS

Zeng Cheng: Conceptualization; visualization; writing – original draft; writing – review and editing. **maurice O'Sullivan:** Supervision; writing – review and editing. **Song Miao:** Funding acquisition; writing – review and editing. **Joseph P. Kerry:** Supervision; writing – review and editing. **Kieran Noel Kilcawley:** Conceptualization; funding acquisition; resources; supervision; writing – review and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable – no new data generated.

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The impact of pasture and non-pasture diets on the sensory and volatile properties of whole milk powder

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Abstract

This study evaluated the impact of three distinct diets; perennial ryegrass (GRS), perennial ryegrass/white clover (CLV) and total mixed ration (TMR), on the sensory properties and volatile profile of whole milk powder (WMP). The samples were evaluated using a hedonic sensory acceptance test ($n = 99$ consumers) and by optimised descriptive profiling (ODP) using trained assessors ($n = 33$). Volatile profiling was achieved by gas chromatography mass spectrometry using three different extraction techniques; headspace solid phase micro-extraction, thermal desorption and high capacity sorptive extraction. Significant differences were evident in both sensory perception and the volatile profiles of the WMP based on the diet, with WMP from GRS and CLV more similar than WMP from TMR. Consumers scored WMP from CLV diets highest for overall acceptability, flavour and quality, and WMP from TMR diets highest for cooked flavour and aftertaste. ODP analysis found that WMP from TMR diets had greater caramelised flavour, sweet aroma and sweet taste, and that WMP from GRS diets had greater cooked aroma and cooked flavour, with WMP derived from CLV diets having greater scores for liking of colour and creamy aroma. Sixty four VOCs were identified, twenty six were found to vary significantly based on diet and seventeen of these were derived from fatty acids; lactones, alcohols, aldehydes, ketones and esters. The abundance of δ-decalactone and δ-dodecalactone was very high in WMP derived from CLV and GRS diets as was γ-dodecalactone derived from a TMR diet. These lactones appeared to influence sweet, creamy, and caramelised attributes in the resultant WMP samples. The differences in these VOC derived from lipids due to diet are probably further exacerbated by the thermal treatments used in WMP manufacture.

Whole milk powder (WMP) contains 26 to 40% (w/w) fat, and is generally manufactured from raw milk and processed by pasteurisation, concentration, evaporation and spray-drying. WMP is an important ingredient for a wide range of food products as it can be reconstituted to produce milk drinks, infant milk formula, yoghurts, milk chocolate and ice cream, amongst others (USDEC, 2005).

WMP sensory characteristics can be influenced by animal diet, heat treatment and other processing and storage conditions (water activity, moisture, packaging, light and temperature: Baldwin *et al.*, 1991; Birchal *et al.*, 2005; Faulkner *et al.*, 2018; Clarke *et al.*, 2020*a*). Pasture-based farming systems are widely practiced in Ireland for the majority of lactation, allowing for the creation of a low-cost, animal welfare friendly, natural feed source to produce high-quality milk products, which are considered more organic and healthier by consumers (Whelan *et al.*, 2017). However, feeding concentrates, such as total mixed ration (TMR) and housing cows indoors is widely implemented in most developed countries mainly for economic reasons (Haskell *et al.*, 2006). Numerous studies have explored the composition of milk from different breeds and feeding systems which have demonstrated that pasture-derived milks have higher levels of polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) and lower levels of saturated fatty acids compared with those derived from TMR diets (O'Callaghan *et al.*, 2019; Kalač and Samková, 2010). A diet of fresh pasture significantly increases levels of β-carotene, enhancing a yellow colour, particularly obvious in butter products derived from pasture, but also apparent in milk and milk powders. Some studies have shown that volatiles generated indirectly through rumen metabolism from forage can also have a sensory impact on milk. For example, *p*-cresol probably contributes to the 'barn-yard'

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aroma/flavour of cow milk derived from pasture (Faulkner *et al.*, 2018; Kilcawley *et al.*, 2018) and it is likely that some volatiles are directly transferred from diet (Villeneuve *et al.*, 2013).

Thermal processing conditions can also alter the volatile profile of milk products. For example, increases of aldehydes and methyl ketones derived from lipid oxidation (decarboxylation or light induced oxidation) of β-keto acids after heat treatment (Vazquez-landaverde *et al.*, 2006; Hougaard *et al.*, 2011), sulphur compounds formed by oxidation of methanethiol (Contarini *et al.*, 1997), esters from heat-catalysed esterification reactions (Faulkner *et al.*, 2018), increases in γ-lactones derived from hydroxy fatty acids after heat processing (Yoshinaga *et al.*, 2019), increases in ketones formed by β-oxidation of saturated fatty acids (Li *et al.*, 2012), Maillard reaction products such as benzaldehyde, furans, maltol, acetaldehyde, 3-methylbutanal, 2-methylbutanal, 2-methylpropanal and possibly acetophenone (Calvo and de la Hoz, 1992) and the degradation of β-carotene resulting in the formation of toluene and xylenes (Zepka *et al.*, 2014). Several ketones, aldehydes and sulphur compounds have been reported to increase during ultra-high temperature treatment and sterilisation of milk (Soukoulis *et al.*, 2007; Al-Attabi *et al.*, 2009; Zabbia *et al.*, 2012). Many of these VOCs can be perceived as off-flavours and can be problematic in products such as WMP, and potentially even carry through into the final product applications resulting in consumer complaints (Hough *et al.*, 2002). Storage time was also shown to have an effect on losses of VOCs in milk powders and this could be due to metabolic and enzymatic reactions post pasteurisation, or chemical reactions (Contarini *et al.*, 1997; Clarke *et al.*, 2020*b*).

Therefore, VOC and sensory profiling of milk and milk products, especially powders such as WMP, is necessary for quality and shelf life purposes. Sensory profiling when undertaken in association with VOC analysis can provide useful additional information in relation to the association between VOCs and sensory properties. VOC profiling is predominately undertaken using gas chromatography mass spectrometry (GC-MS) and recent studies have shown the importance of extraction method choice in relation to VOC profiling of WMP (Cheng *et al.*, 2021) and the impact of diet on the oxidative and sensory shelf life of WMP (Clarke *et al.*, 2021). Three distinct VOC extraction techniques were utilised in this study in order to obtain a more complete VOC profile; headspace solid phase micro-extraction (HS-SPME), thermal desorption (TD) and a high capacity sorptive extraction (Hi-Sorb) procedure by direct immersion (DI). The aim of this study was to investigate the effect of three distinct diets (GRS, CLV and TMR) on the sensory and VOC profile of WMP.

Materials and methods

Whole milk powder manufacture

Raw milk from 54 Friesian cows was split into 3 groups ($n = 18$) at the Teagasc Moorepark dairy farm, Teagasc, Animal & Grassland Research Centre, Fermoy, Co. Cork, Ireland. Each group of 18 cows were given separate diets; outdoor pasture grazing on perennial ryegrass (*Lolium perenne*) denoted as GRS, outdoor pasture grazing on perennial ryegrass supplemented with white clover (*Trifolium repens*) denoted as CLV, and indoors on total mixed rations (TMR) consisting of grass silage, maize silage and concentrates, which was a replication of the study outlined in detail by O'Callaghan *et al.* (2016). Raw whole milk (approximately 1000 kg) was collected from cows on each dietary treatment. The preparation of the WMP was as outlined in Cheng *et al.* (2021). The milk was not standardised, but the average fat contents of the milk from each diet in triplicate were quite similar (GRS 27.9%, CLV 28.2%, TMR 28.2%). WMP samples were stored at room temperature in sealed 900 g aluminium vacuum cans until analysis (all analysis was undertaken within the designated shelf life of the samples).

Sample preparation

Prior to sensory and VOC analysis WMP from each dietary treatment (GRS, CLV and TMR) at <2 months was dissolved at 10% solids (w/v) using ultra-pure deionised water and stored at 4°C overnight to ensure solubility without overhead lights to prevent light-induced off-flavour formation as outlined in Cheng *et al.* (2020) in the preparation of SMP for sensory analysis. VOC analysis and sensory analysis were performed the following day.

To monitor the performance of each extraction procedure, an internal standard (IS) of 2-phenyl-D5-ethanol and 4-methyl-2-pentanol (Merck., Arklow, Wicklow, Ireland) at 20 mg/l in ultra-pure water, was added (50 µl) to each WMP sample prior to extraction.

Volatile organic compound extraction

The extraction procedures for HS-SPME, TD and DI-HiSorb were identical to those described by Cheng *et al.* (2021).

GC–MS analysis

The GC–MS system was an Agilent 7090A GC and Agilent 5977B MSD (Agilent Technologies Ltd., Cork, Ireland) using a non-polar column DB5 (60 m × 0.25 mm × 0.25 µm) (Agilent Technologies Ltd., Ireland). The column oven was held at 40°C for 5 min, then increased to 230°C at 5°C/min and held at 230°C for 35 min, yielding a total run time of 60 min. The carrier gas was helium held at a constant flow of 1.2 ml/min. The ion source temperature was 220°C and the interface temperature was set at 260°C. The mass spectrometer was in electronic ionisation (70 V) mode with the mass range scanned between 35 and 250 amu. Analysis was undertaken using Mass Hunter Qualitative Analysis Software (Agilent Technologies Ltd) with target and qualifier ions and linear retention indices for each compound compared an in-house library based on mass spectra obtained from NIST 2014 mass spectral library MS searching (v.2.3, Gaithersburg, MD, USA), and an in-house library created using authentic compounds with target and qualifier ions and linear retention indices for each compound using Kovats index. Spectral deconvolution was also performed to confirm identification of compounds using Automated Mass spectral Deconvolution and Identification System (AMDIS). Batch processing of samples was carried out using MetaMS (Wehrens *et al.*, 2014). MetaMS is an open-source pipeline for GC–MS-based untargeted metabolomics. The peak areas of the analytes were normalised to the peak areas of the IS (4-methyl-2-penanol) at first and then expressed as a percentage of the total.

To monitor the performance of the GC-MS operating conditions, an external standard (ES) solution was added at the start and end of each GC-MS sample run, the peak areas were monitored to ensure they were within a specified tolerance (10% coefficient of variation) to ensure that both the extraction and MS detection were performing within specification during the

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analysis. The ES was comprised of 1-butanol, dimethyl disulphide, butyl acetate, cyclohexanone, and benzaldehyde (Merck, Ireland) at 10 mg/l with 2-phenyl-D5-ethanol (Merck, Arklow, Co., Wicklow, Ireland) added at 5 mg/l in ultra-pure water. For the HS-SPME technique, 10 µl of ES was added to the sample in a 20 ml amber HS- SPME vial (Apex Scientific Ltd., Maynooth, Ireland). For TD and DI-HiSorb, the ES (10 µl) was added to the TD tube containing the sample extract for both TD and DI-HiSorb extracts.

Sensory analyses

All sensory analyses was undertaken at the sensory facility within the School of Food and Nutritional Science, University College Cork, Co. Cork, Ireland, according to International Standards (ISO 11136, 2014).

Milk consumers residing in Cork (Ireland, $n = 99$) (70 : 30 male/female, age 18–50 years), participated in the consumer test (hedonic attribute testing). The consumers consisted of students and staff from Sensory Group, School of Food and Nutritional Science, University College Cork, Co. Cork, Ireland. Consumers were regular self-reported consumers of milk, had experiences in drinking powdered milk products, and were non-rejecters of milk. Participants used the sensory hedonic descriptors (Table 1) provided to them for three different WMP samples (CLV, GRS and TMR) presented in triplicate at 10% solids (w/v). For consumer testing, samples were dispensed into 30 ml inert plastic tumblers provided with three digit codes presented simultaneously but with randomised order to prevent first order and carry-over effects (Macfie *et al.*, 1989). Samples were taken from the refrigerator (4°C) and served after 15 min at 12°C temperature. Participants were first asked to evaluate the overall appearance and colour of the sample. The appearance of each sample was scaled using a 1–9 hedonic scale, where 1 = dislike extremely and 9 = like extremely. They were then asked to taste the sample and evaluate their overall impression using a 9-point

Table 1. Sensory terms for the affective (consumer acceptance testing) and optimised descriptive profiling (ODP) of whole milk powder

Descriptor	Explanation	Scale
Consumer Acceptance Testing		
Appearance-Liking	The liking of appearance	0 = extremely dislike 10 = extremely like
Flavour-Liking	The liking of flavour	0 = extremely dislike 10 = extremely like
Aroma-Liking	The liking of aroma	0 = extremely dislike 10 = extremely like
Texture-Liking	The liking of texture	0 = extremely dislike 10 = extremely like
Overall acceptability	The acceptability of the product	0 = extremely unacceptable 10 = extremely acceptable
Colour-Liking	The liking of colour	0 = extremely dislike 10 = extremely like
Optimised Descriptive Profiling		
Appearance-colour	Appearance-Ivory to orange colour	0 = Pale, 10 = Yellow
Sweet aroma	The smell associated with dairy sweet milky products	0 = none, 10 = extreme
Creamy aroma	The smell associated with creamy/milky products	0 = none, 10 = extreme
Cooked aroma	The smell associated with cooked milk products	0 = none, 10 = extreme
Oxidised aroma	The smell associated with rancid or oxidised products	0 = none, 10 = extreme
Painty aroma	The smell associated with rancid paint type notes	0 = none, 10 = extreme
Chalky texture	Chalk like texture in the mouth	0 = none, 10 = extreme
Powdery texture	Powdery texture in the mouth	0 = none, 10 = extreme
Viscosity	Thick texture in the mouth	0 = none, 10 = extreme
Sweet taste	Fundamental taste sensation of which sucrose is typical	0 = none, 10 = extreme
Cream flavour	The flavour associated with creamy/milky products	0 = none, 10 = extreme
Dairy sweet flavour	The flavours associated with sweetened cultured dairy products such as fruit yoghurt	0 = none, 10 = extreme
Dairy fat flavour	Intensity of fat flavour	0 = none, 10 = extreme
Oxidised flavour	The flavour associated with rancid or oxidised products	0 = none, 10 = extreme
Rancid butter	The flavour associated with rancid or oxidised butter	0 = none, 10 = extreme
Painty flavour	The flavour associated with rancid paint type notes	0 = none, 10 = extreme
Fruity/Estery flavour	The flavours associated with fatty acid ethyl esters	0 = none, 10 = extreme
Cooked flavour	The flavour associated with cooked milk products	0 = none, 10 = extreme
Off-flavour	Off-flavour (Rancid)	0 = none, 10 = extreme
Astringent after-taste	Fundamental taste sensation of which aluminium sulphate is typical	0 = none, 10 = extreme

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hedonic scale. The assessors were asked to assess using a 9-point hedonic scale the liking of flavour, freshness, liking of appearance, liking of aroma, overall acceptability, cooked flavour, thickness, creaminess, aftertaste, intensity of aftertaste and quality.

Optimised descriptive profiling (ODP: da Silva *et al*., 2012, 2013) was undertaken with trained panellists at University College Cork, Ireland ($n = 33$). These assessors were presented with all samples simultaneously, but with randomised order to prevent first order and carry-over effects (Macfie *et al*., 1989). Assessors used the consensus list of sensory descriptors as described by Cheng *et al*. (2020) which were measured on a 10 cm line scale with the term 'none' used as the anchor point for the 0 cm end of the scale and 'extreme' for the 10 cm end of the scale (Table 1). Sensory terms, which were the main sensory dimensions, were pre-selected from the sample set using an expert sensory panel ($n = 10$). Assessors evaluated the intensity of each attribute for each sample on the scales. Attributes were presented along with the table describing the sensory terms (Table 1). All samples were prepared in the same manner as the consumer analysis study and presented in duplicate.

Statistical analysis

Statistical analyses for data relating to volatile analysis were carried out using one way-ANOVA. The level of significance for correlation was set at $P < 0.05$. To classify WMP samples in a supervised multivariate model, partial least-squares discriminant analysis (PLS-DA) was performed. Unsupervised hierarchical clustering analysis (HCA) was used to show the patterns in the VOC profile and is presented as a heatmap (Online Supplementary Fig. S1). Consumer acceptance data obtained from sensory analysis was evaluated by one-way ANOVA using differences of perception related to diet as the primary factors. Analyses were carried out at only one time point and where normally distributed were analysed using one-way ANOVA with post hoc. The SPSS V23.0 (IBM Statistics Inc., Armonk, NY) was used for one-way ANOVA. Principal component analysis (PCA) plots of the volatile and diet data were used to demonstrate correlations between the VOC and the different diet attributes. These were constructed using the 'factoextra' and 'FactoMinoR' packages in R (v 3.4.1). The Unscrambler X software, version 10.3 (Camo Software, Oslo, Norway) was used for ANOVA-PSLR (APLSR) analysis of WMP data from different diets and variance of OPD sensory data. Analysis of variance (ANOVA) with post hoc Tukey significant test was applied to OPD data, working at an alpha level of 0.05. The correlations between sensory attributes and VOC were also analysed by PLSR. PLSR were performed with VOC data as the X-matrix and sensory attributes as the Y-matrix. Regression coefficients were analysed by Jack-knifing to derive significance indicators for the relationships determined in the quantitative APLSR (data not shown). Metabolic Analyst 4.0 (McGill University, Montreal, QC, Canada) software was used to perform the HCA graph (Chong *et al*., 2018).

Results

Volatile analysis

The volatile compounds identified in the WMP from the three different diets (GRS, CLV and TMR), using three extraction methods are listed in Table 2. Results are expressed as percentage abundance of each VOC per extraction method based on the recovery in relation to the recovery of the IS for that extraction method. A total of 64 VOCs were identified in these WMP derived from all three diets by HS-SPME, TD and DI-HiSorb. Distinct differences in the VOCs profiles were evident between the different extraction techniques, which highlights the effectiveness of each extraction technique for particular chemical classes. The same trends in relation to individual VOCs were evident across the different extraction techniques where a VOC was identified by one or more extraction technique. Eight lactones, 5 aldehydes, 3 ketones, 3 terpenes, 2 alcohols, 2 esters, 1 acid, 1 hydrocarbon and 1 sulphur compound varied significantly ($P < 0.05$ or $P < 0.01$) in the WMP based on cow diet (Table 2).

Seven VOCs were significantly more abundant in WMP derived from CLV; 1-pentanol ($P < 0.05$) and 3-methyl-butanal, δ-octalactone, α-pinene, 3-carene, acetic acid and ethyl-benene ($P < 0.01$). Four VOCs were significantly more abundant in the WMP derived from GRS; methyl-hexanoate ($P < 0.05$) and butanal, 2,3-octanedione and 2-nonanone ($P < 0.01$). Five VOCs were significantly more abundant in the WMP derived from TMR; γ-dodecalactone GRS ($P < 0.05$) and dimethyl sulphide, D-limonene, heptanal and o-xylene ($P < 0.01$). Another eight VOCs were significantly more abundant in WMP derived from both CLV and GRS in comparison to WMP derived from TMR; δ-decalactone and δ-dodecalactone ($P < 0.05$) and δ-tridecalactone, hexanol, hexanal, δ-hexalactone, δ-nonalactone and δ-undecalactone ($P < 0.01$). Another VOC, pentanal, was significantly ($P < 0.01$) more abundant in WMP derived from GRS in comparison to WMP from TMR, but was not statistically different to WMP derived from CLV (nor was there any statistical difference between WMP from CLV or TMR). Similarly diacetyl was significantly higher ($P < 0.01$) in WMP derived from CLV than from WMP derived from TMR, but was not statistically different to WMP from GRS (WMP from GRS and from TMR were also not statistically different). These associations are more clearly represented in the PCA plots (Fig. 1a and b) and by the HCA heatmap (online Supplementary Fig. S1), where it is evident that both the WMP derived from GRS and CLV diets are more comparable, but distinctly separate from WMP derived from TMR diets (Fig. 1a and b).

Sensory evaluation

The sensory attributes identified in the WMP derived from CLV, GRS and TMR diets by consumer acceptance are shown in Fig. 2. Five significant sensory differences ($P < 0.05$) were observed between the WMP derived from these diets; creaminess, aftertaste, cooked-flavour, quality and liking of flavour. The WMP derived from CLV diets scored statistically ($P < 0.05$) highest for liking of flavour, creaminess and quality. The WMP derived from TMR diets scored significantly highest ($P < 0.05$) for cooked-flavour and aftertaste. The WMP derived from GRS diets did not score significantly different for any of the sensory attributes in comparison to WMP samples derived from either CLV or TMR diets.

The ODP evaluation of WMP from different diets is shown in the PCA plot (online Supplementary Fig. S2). The significance (*P*-value) of regression coefficients and average results (ANOVA) for the ODP attributes for WMP from different diets (CLV, GRS and TMR) are illustrated in Table 3. The assessors rated WMP derived from CLV diets as significantly greater for liking of colour ($P < 0.05$) and creamy aroma ($P < 0.05$) in comparison to the WMP-derived TMR diets. The WMP derived from

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Table 2. The volatile compounds in the whole milk powders derived from different diets, grass (GRS), grass/clover (CLV) and total mixed ration (TMR) by GCMS from three volatile extraction procedures

			DI-HiSorb				TD				HS-SPME			
Compounds	CAS no.	REF RI	CLV	GRS	TMR	*P*-value	CLV	GRS	TMR	*P*-value	CLV	GRS	TMR	*P*-value
Alcohol														
Ethanol	64-17-5	426	0.34	0.42	0.05	NS	ND	ND	ND		ND	ND	ND	
1-Pentanol	71-41-0	768	0.65^{a}	0.55^{b}	0.44^{c}	***	3.03^{a}	2.03^{b}	1.13^{c}	***	0.68^{a}	0.61^{b}	0.33^{c}	***
1-Hexanol	111-27-3	868	ND	ND	ND		0.86^{a}	0.67^{a}	0.47^{b}	*	0.71^{a}	0.52^{a}	0.11^{b}	*
1-Hexanol,2-ethyl	104-76-7	1030	1.45	1.36	1.17	NS	8.10	6.94	6.50	NS	ND	ND	ND	
α-Terpineol	10482-56-1	1192	ND	ND	ND		0.26	0.25	0.27	NS	ND	ND	ND	
Aldehyde														
Acrolein	107-02-8	470	1.95	1.59	2.10	NS	ND	ND	ND		ND	ND	ND	
Butanal	123-72-8	596	0.76^{b}	1.53^{a}	0.50^{b}	*	ND	ND	ND		ND	ND	ND	
Butanal, 3-methyl-	590-86-3	654	ND	ND	ND		3.65^{a}	2.47^{b}	2.03^{c}	*	7.99^{a}	5.59^{b}	2.46^{c}	*
Pentanal	110-62-3	697	0.38^{ab}	0.43^{a}	0.21^{b}	*	ND	ND	ND		ND	ND	ND	
Hexanal	66-25-1	801	6.45^{a}	6.21^{a}	2.34^{b}	*	13.65^{a}	11.3^{a}	3.13^{b}	*	10.9^{a}	9.40^{a}	2.97^{b}	*
Heptanal	111-71-7	901	2.54^{b}	2.66^{b}	5.03^{a}	*	20.5^{b}	20.20^{b}	24.98^{a}	*	8.53^{b}	8.45^{b}	12.69^{a}	*
Benzaldehyde	100-52-7	960	0.87	1.11	1.40	NS	1.38	1.31	2.74	NS	0.43	0.34	0.45	NS
Octanal	124-13-0	1004	1.29	1.31	2.40	NS	4.16	2.90	4.22	NS	0.80	0.42	0.51	NS
Benzeneacetaldehyde	122-78-1	1048	0.46	0.43	0.33	NS	ND	ND	ND		ND	ND	ND	
2-Octenal,(E)-	2548-87-0	1057	0.61	0.55	0.42	NS	0.29	0.18	0.43	NS	ND	ND	ND	
Nonanal	124-19-6	1106	6.07	6.20	7.32	NS	17.67	21.10	16.01	NS	1.00	1.04	1.08	NS
2-Nonenal,(E)-	18829-56-6	1160	0.38	0.46	0.45	NS	0.23	0.18	0.19	NS	ND	ND	ND	
Decanal	112-31-2	1205	1.76	1.43	1.38	NS	1.64	2.44	1.18	NS	ND	ND	ND	
2-Decenal,(E)-	3913-81-3	1266	0.24	0.31	0.35	NS	0.14	0.14	0.35	NS	ND	ND	ND	
Undecanal	112-44-7	1309	0.21	0.28	0.29	NS	0.21	0.25	0.15	NS	ND	ND	ND	
Dodecanal	112-54-9	1401	0.43	0.56	0.67	NS	0.14	0.27	0.47	NS	ND	ND	ND	
Hydrocarbons														
Benzene	71-43-2	669	1.18	1.55	1.18	NS	6.10	11.62	6.40	NS	2.52	2.27	1.14	NS
Toluene	108-88-3	763	0.35	0.61	0.42	NS	3.97	4.21	2.74	NS	0.40	0.86	0.24	NS
p-Xylene	106-42-3	867	ND	ND	ND		2.52	2.99	3.90	NS	ND	ND	ND	
o-Xylene	95-47-6	900	ND	ND	ND		1.01^{b}	1.09^{b}	1.58^{a}	*	ND	ND	ND	
Phenol	108-95-2	995	0.70	0.67	0.63	NS	0.65	0.70	0.64	NS	ND	ND	ND	

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Benzyl alcohol	100-51-6	1037	ND	ND	ND		0.26	0.17	0.25	NS	ND	ND	ND	
Ketone														
Diacetyl	431-03-8	596	ND	ND	ND		0.36^{a}	0.33^{ab}	0.24^{b}	*	ND	ND	ND	
Hydroxyacetone	116-09-6	657	1.36	0.64	1.09	NS	ND	ND	ND	ND	ND	ND		
2-Pentanone	107-87-9	687	1.53	1.41	1.19	NS	6.94	7.70	8.47	NS	5.25	6.29	5.02	NS
2-Butanone	108-10-1	740	ND	ND	ND		7.30	7.10	7.49	NS	1.79	1.35	1.11	NS
Methyl Isobutyl Ketone	108-10-1	740	0.53	0.49	0.43	NS	1.07	0.87	0.84	NS	0.15	0.33	0.23	NS
2-Heptanone	110-43-0	891	6.26	6.28	6.06	NS	7.29	8.92	9.69	NS	17.00	19.1	12.38	NS
2,3-Octanedione	585-25-1	967	0.17^{b}	0.19^{a}	0.11^{c}	*	ND	ND	ND	ND	ND	ND		
2-Octanone	111-13-7	992	0.38	0.38	0.37	NS	0.60	0.36	0.68	NS	ND	ND	ND	
3,5-Octadien-2-one	38284-27-4	1072	ND	ND	ND		0.50	0.60	0.90	NS	0.21	0.10	0.13	NS
2-Nonanone	821-55-6	1094	2.76^{b}	2.83^{a}	2.69^{b}	*	1.31^{b}	1.79^{a}	1.63^{b}	*	2.32^{b}	2.54^{a}	2.13^{b}	*
2-Undecanone	112-12-9	1294	0.47	0.40	0.40	NS	0.16	0.14	0.14	NS	ND	ND	ND	
2-Tridecanone	593-08-8	1480	0.34	0.31	0.15	NS	ND	ND	ND	ND	ND	ND		
2-Pentadecanone	2345-28-0	1689	0.55	0.47	0.18	NS	ND	ND	ND	ND	ND	ND		
2-Heptadecanone	2922-51-2	1878	0.56	0.61	0.46	NS	ND	ND	ND	ND	ND	ND		
Lactone														
γ-Crotonolactone	497-23-4	916	0.12	0.27	0.17	NS	ND	ND	ND	ND	ND	ND		
δ-Hexalactone	823-22-3	1084	0.11^{a}	0.12^{a}	0.06^{b}	*	ND	ND	ND	ND	ND	ND		
δ-Octalactone	698-76-0	1288	0.92^{a}	0.75^{b}	0.23^{c}	*	ND	ND	ND	ND	ND	ND		
δ-Nonalactone	3301-94-8	1404	0.15^{a}	0.16^{a}	0.09^{b}	*	ND	ND	ND	ND	ND	ND		
δ-Decalactone	705-86-2	1506	31.00^{a}	32.10^{a}	21.90^{b}	***	ND	ND	ND	0.43^{a}	0.38^{a}	0.28^{b}	***	
δ-Undecalactone	710-04-3	1627	0.21^{a}	0.20^{a}	0.08^{b}	*	ND	ND	ND	ND	ND	ND		
γ-Dodecalactone	2305-(05)-7	1674	4.20^{b}	2.80^{c}	34.64^{a}	***	ND	ND	ND	ND	ND	ND		
δ-Dodecalactone	713-95-1	1719	44.50^{a}	45.99^{a}	29.64^{b}	***	ND	ND	ND	ND	ND	ND		
δ-Tridecalactone	7370-92-5	1778	1.45^{a}	1.46^{a}	0.72^{b}	*	ND	ND	ND	ND	ND	ND		
Sulphur compounds														
Dimethyl sulphide	75-18-3	510	ND	ND	ND		ND	ND	ND	1.00^{b}	1.61^{b}	2.42^{a}	*	
Dimethyl disulphide	624-92-0	739	0.55	0.51	0.50	NS	5.16	4.21	4.48	NS	0.95	0.41	0.29	NS
Terpenes														
α-Pinene	80-56-8	930	ND	ND	ND		ND	ND	ND	3.28^{a}	1.65^{b}	0.86^{c}	*	
3-Carene	13466-78-9	1009	ND	ND	ND		ND	ND	ND	2.92^{a}	1.74^{b}	0.89^{c}	*	
D-Limonene	5989-27-5	1022	0.25^{b}	0.25^{b}	0.54^{a}	*	0.53^{b}	0.67^{b}	2.34^{a}	*	0.14^{b}	0.08^{b}	0.37^{a}	*

(*Continued*)

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Table 2. (*Continued.*)

			DI-HiSorb				TD				HS-SPME			
Compounds	CAS no.	REF RI	CLV	GRS	TMR	*P*-value	CLV	GRS	TMR	*P*-value	CLV	GRS	TMR	*P*-value
Longifolene	475-20-7	1432	ND	ND	ND		0.23	0.13	0.13	NS	ND	ND	ND	
Acids														
Acetic acid	64-19-7	629	0.37^{a}	0.17^{b}	0.15^{b}	*	ND	ND	ND	ND	ND	ND		
Esters														
Methyl butanote	623-42-7	724	ND	ND	ND		2.02	2.03	1.93	NS	1.81	2.30	3.19	NS
Methyl pyruvate	108-10-1	740	0.12	0.20	0.14	NS	ND	ND	ND	ND	ND	ND		
Ethylbenzene	100-41-4	851	ND	ND	ND		1.15^{a}	0.42^{b}	0.47^{b}	*	ND	ND	ND	
Methy hexanoate	106-70-7	922	ND	ND	ND		ND	ND	ND	1.49^{c}	5.45^{a}	3.12^{b}	***	
Furans														
Furan,2-methyl-	534-22-5	604	0.97	0.89	0.90	NS	ND	ND	ND	8.82	7.59	6.35	NS	
2-Furanmethanol	98-0-0	850	1.04	0.36	0.38	NS	ND	ND	ND	ND	ND	ND		
Furfural	98-01-1	852	0.56	0.29	0.23	NS	ND	ND	ND	ND	ND	ND		

RI: Retention index. REF RI: Reference retention index. CAS no: Chemical Abstracts Service Number. One-way ANOVA statistical analysis :* and *** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively. Values in the same row not sharing the same superscript (a,b,c) specify significant difference in peak area % value carried out by Tukey post hoc test.
Results are expressed as percentage abundances for each extraction technique based on the recovery of the internal standard (2-phenyl-D5-ethanol).

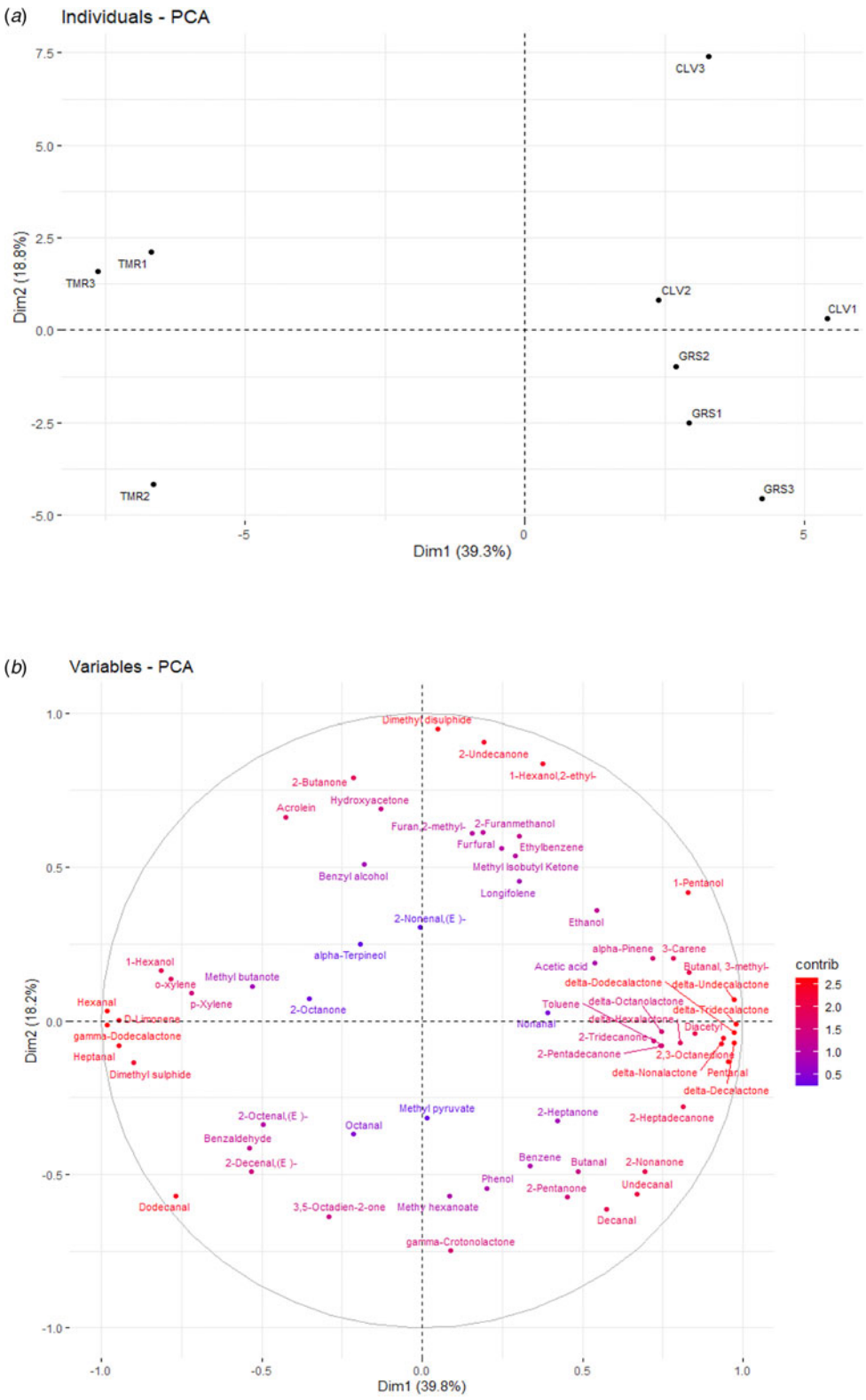

Fig. 1. Principal component analysis: (a) Three kinds of feeding system of grass (GRS), grass/clover (CLV) and total mixed rations (TMR). Scores and loadings are based on the average of three repetitions for each feeding diets. (b) Variables: the relative percent amount of 64 volatile compounds. Colour gradient: low = white, mid = blue and high = red, midpoint set 1.0.

GRS diets scored statistically ($P < 0.01$) higher for cooked aroma and cooked flavour and WMP derived from TMR diets highest for caramelised flavour ($P < 0.05$), sweet aroma and sweet taste ($P < 0.01$).

OPD sensory and volatile data correlations

APLSR was conducted to study the relationships of the individual VOCs with the sensory descriptors as used in the ODP study.

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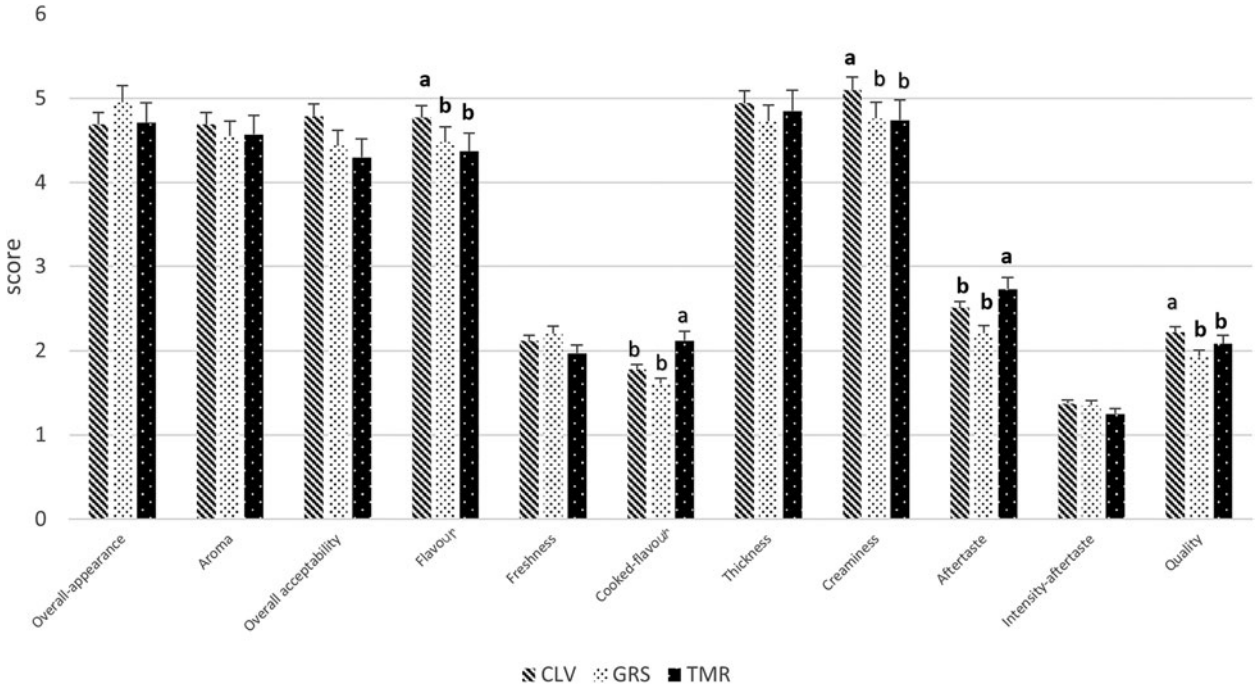

Fig. 2. Hedonic sensory analysis of whole milk powder drink derived from different feeding systems of grass (GRS), grass/clover (CLV) and total mixed rations (TMR). The whole milk powder samples were assessed by consumers ($n = 99$) familiar with milk using blind replicates in a full balanced block design, where consumers evaluated all samples in duplicate. The error bars represent standard mean error within replicates. Columns with different letters (a–c) for each attribute are statistically different ($P < 0.05$) carried out by ANOVA and Tukey post hoc test.

The X-matrix was composed of 64 VOCs, whereas the Y-matrix consisted of the sensory attributes from OPD (Fig. 3). When the two PLSs were taken into account, 63% of the VOCs explained 36% of the variation among the sensory data and diets. All variances were placed between the inner ($r^2 = 0.5$) and outer ellipses ($r^2 = 1.0$), thereby indicating that the APLSR model sufficiently described the associations between descriptors and VOCs. The centre ellipsoid in Fig. 3 indicates 50% of the explained variation. Many VOC were located inside the ellipsoid, which means they did not greatly contribute to the model. Figure 3 demonstrates that the WMP samples are separated along PLS1, with the WMP derived from TMR on the right side and the WMP derived from GRS and CLV on the left side. This highlights again that WMP derived from TMR diets is more distinct than the WMP from both the CLV and GRS diets.

Discussion

The source of many of these VOCs in WMP is varied and probably a combination of some or all of the following; direct transfer from the diet, rumen metabolism, metabolism in the raw milk, created during heat processing or by auto chemical reactions. However, some trends were evident. Seventeen of the twenty six VOCs that were significantly different based on diet in these WMP samples are derived from fatty acids either by lipid oxidation, thermal degradation or β-oxidation and lactonisation (butanal, pentanal, hexanal, hexanal, 1-pentanol, 1-hexanol, 2-nonanone, 2,3-octanedione, δ-hexalactone, δ-octanolactone, δ-nonalactone, δ-decalactone, δ-undecalactone, δ-dodecalactone, δ-tridecalactone, γ-dodecalactone). It is well established that different diets have a significant impact on the fatty acid profile of cow's milk (Kalač and Samková, 2010; O'Callaghan *et al.*, 2016), hence it is logical that VOCs derived from fatty acids are also likely to be significantly impacted. Such differences may be further exacerbated by subsequent thermal treatment during processing to WMP enhancing lipid oxidation and Maillard and lactonisation reactions (Calvo and de la Hoz, 1992; Havemose *et al.*, 2006; Kilcawley *et al.*, 2018; Clarke *et al.*, 2021).

Straight-chain aldehydes are major contributors to off-flavours in dairy products (Barrefors *et al.*, 1995). Previous studies have also found that the abundance of methyl ketones also from lipid oxidation were correlated to the severity of heat treatment in milk and associated with off-flavour development that can be carried over to final product applications (Nursten, 1997). Only one methyl ketone, 2-nonanone was significantly different based on diet, and most abundant in WMP derived from GRS. The ketone 2,3-octanedione, which is also a product of lipid oxidation, has previously been shown to be higher in milk derived from pasture (Coppa *et al.*, 2011) as found in this study. Two primary alcohols, 1-pentanol and 1-hexanol are also products of lipid oxidation. 1-Pentanol is derived from the primary aldehyde pentanal and was significantly correlated with the WMP from CLV diets, in agreement with previous studies on milk (Faulkner *et al.*, 2018; Clarke *et al.*, 2020*a*). Villeneuve *et al.* (2013) found levels of 1-pentanol were significantly higher in milk derived from pasture and silage than from hay, however, other studies have not found an impact of diet on levels of 1-pentanol (Coppa *et al.*, 2011). 1-Hexanol, derived from hexanal (Zhang *et al.*, 2016) was significantly higher in the WMP derived from TMR diets, in agreement with that found by Faulkner *et al.* (2018) and Clarke *et al.* (2020b) in milk, but other studies did not find an impact of diet on levels of 1-hexanol in milk (Villeneuve *et al.*, 2013). Esters are probably present as a result of microbial activity in the rumen, post-pasteurisation microbial contamination or

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Table 3. ODP (Optimised Descriptive Profiling) evaluation by assessors of whole milk powder produced by different diets, grass (GRS), grass/clover (CLV) and total mixed rations (TMR)

	Beta Coefficients			ANOVA			
	CLV	GRS	TMR	CLV	GRS	TMR	*P*-value
Colour	0.174***	0.116*	−0.290	5.47[a]	5.78[a]	3.18[b]	***
Sweet aroma	−0.037	−0.025	0.062	4.07[c]	4.57[b]	4.85[a]	*
Creamy aroma	0.038	0.025	−0.064	4.41[a]	4.03[b]	3.72[b]	*
Cooked aroma	0.036	0.024	−0.061	3.74[b]	4.32[a]	3.54[b]	*
Oxidised aroma	0.017	0.011	−0.029	1.86	2.10	1.73	NS
Painty Aroma	0.002	0.001	−0.003	1.38	1.39	1.47	NS
Chalky texture	−0.010	−0.007	0.017	3.06	3.10	3.23	NS
Powdery texture	−0.040	−0.026	0.066	2.99	3.47	3.80	NS
Viscosity	−0.013	−0.009	0.022	4.23	4.19	4.39	NS
Sweet taste	−0.032	−0.021	0.054	5.00[b]	4.97[b]	5.53[a]	*
Sour taste	−0.016	−0.010	0.026	1.94	2.05	2.20	NS
Salty taste	−0.009	−0.006	0.015	1.62	1.48	1.67	NS
Creamy flavour	−0.034	−0.023	0.057	5.12	5.08	5.59	NS
Dairy sweet flavour	−0.015	−0.010	0.024	5.02	4.84	5.18	NS
Carmelised flavour	−0.061*	−0.040 *	0.101	2.95[b]	3.09[b]	4.03[a]	***
Oxidised flavour	−0.012	−0.008	0.020	2.26	2.50	2.53	NS
Rancid butter	−0.021	−0.014	0.036	1.95	2.01	2.27	NS
Painty flavour	0.009	0.006	−0.016	1.64	1.61	1.51	NS
Grassy/Hay	0.014	0.009	−0.023	2.46	2.47	2.27	NS
Cooked flavour	0.005	0.003	−0.008	5.84[ab]	7.67[a]	4.81[b]	*
Off flavour	−0.001	−0.001	0.001	2.08	2.08	2.10	NS
Astringency	−0.009	−0.006	0.014	1.80	1.56	1.80	NS

ANOVA values are the average results. One-way ANOVA statistical analysis :* and *** denote significant differences at $P < 0.05$ and $P < 0.01$, respectively. Values in the same row not sharing the same superscript (a, b,c) specify significant difference in sensory attribute value carried out by Tukey post hoc test.
Correlation relationships and ANOVA analysis with sensory attributes observed in whole milk powder. Positive and negative values indicate positive and negative correlations between the diets and sensory attributes, respectively.

heat-catalysed esterification during thermal-treatment (Manzocchi *et al.*, 2021; Vazquez-Landaverde *et al.*, 2006). Only two fatty acid esters were found in these WMP samples and only methyl hexanoate varied with diet. Methyl hexanoate was found to be significantly more abundant in WMP derived from GRS diets.

The significant difference in the abundance and presence of lactones in the WMP derived from these diets is especially interesting due to their potential positive sensory impact (the abundance of 8 of the 9 lactones identified varied with diet). Lactones are cyclic compounds, formed through metabolism and/ or thermal degradation of δ- and γ-hydroxyacids and are known to contribute to sweet, creamy and fatty flavours in milk powders (Yoshinaga *et al.*, 2019; Ianni *et al.*, 2020). The recovery of lactones was mostly achieved using the DI-HiSorb extraction method, which is a sorptive extraction technique particularly useful for extracting lactones in contrast to other extraction methods (High *et al.*, 2019; Cheng *et al.*, 2021). Some studies have stated that milk from cows fed hay (timothy) was characterised by higher contents of γ-lactones than silage-fed and pasture-fed derived milk (Villeneuve *et al.*, 2013; Bovolenta *et al.*, 2014). This may be why γ-dodecalactone is significantly more abundant in WMP from TMR in this study. The proliferation of both δ-decalactone and δ-dodecalactone in these samples, but especially in WMP derived from CLV and GRS diets, might also relate to changes in the fatty acid profile and hydroxy acid content due to the different diets (Villeneuve *et al.*, 2013; Ianni *et al.*, 2020). As thermal treatments increase the levels of free hydroxy acids it may be assumed that dietary differences in lactone contents are exacerbated during the spray drying process for the preparation of the WMP (Calvo and de la Hoz, 1992).

The remaining VOC that were significantly different based on diet and not derived from lipids were 3-methyl-butanal, dimethyl sulphide, α-pinene, 3-carene, D-limonene, ethylbenzene, *o*-xylene, acetic acid, and diacetyl. The only Strecker degradation product was 3-methyl-butanal which was significantly more abundant in WMP derived from CLV diets and is a product of leucine metabolism, or also possibly directly transferred from the diet (Faulkner *et al.*, 2018). Strecker derived VOCs can also be involved in the Maillard reaction, and some have previously been shown to be indicators of severely heat-treated milk and UHT milk (Calvo and de la Hoz, 1992; Belitz *et al.*, 2004). Previous studies have identified 3-methyl-butanal in milk, but it

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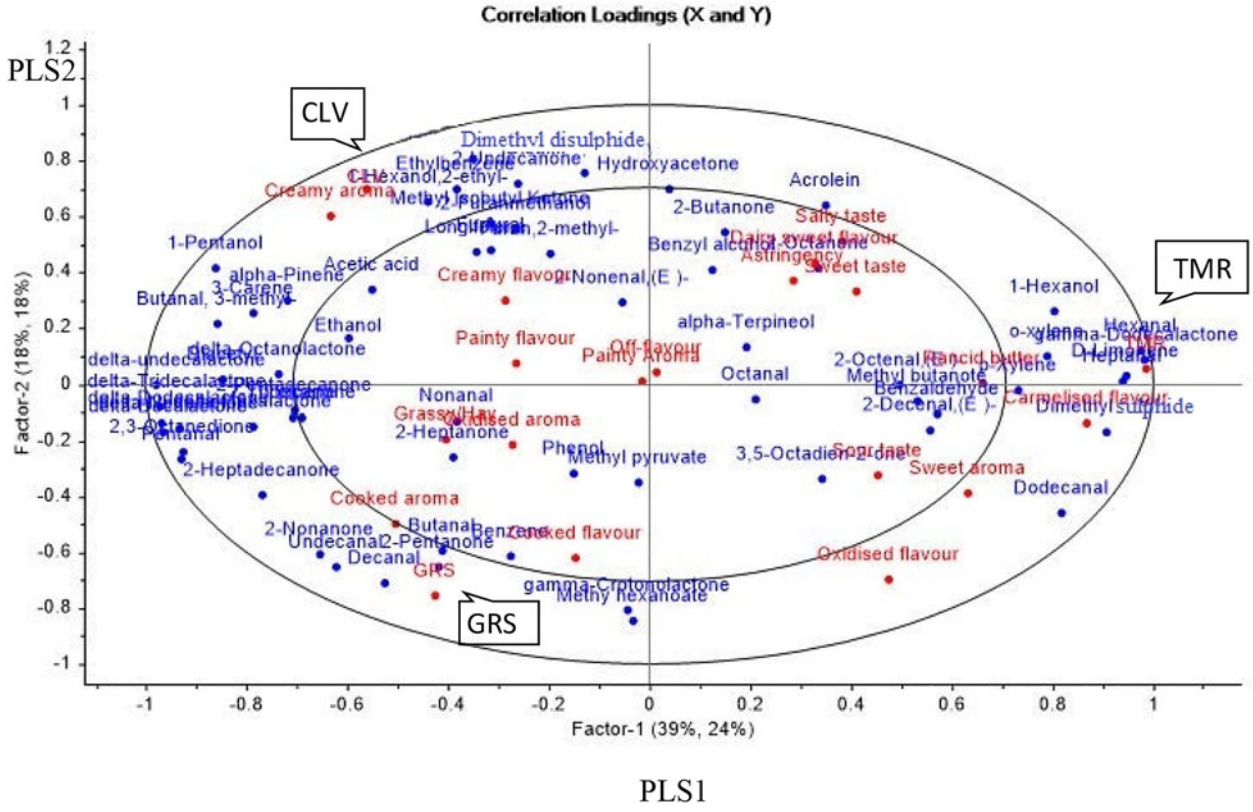

Fig. 3. ANOVA-PLSR correlation loadings plot of sensory attributes (aroma and flavour) by OPD analysis and volatile compounds (X-matrix) in the WMP from the three distinct diets, grass (GRS), grass/clover (CLV) and total mixed rations (TMR). Ellipses represent $r^2 = 0.5$ and 1.0, respectively.

was not impacted by diet (Bendall, 2001; Faulkner *et al.*, 2018). Dimethyl sulphide was significantly higher in WMP derived from TMR than from GRS or CLV diets. Dimethyl sulphide is mainly derived from methionine, but the impact of diet is unclear (Clarke *et al.*, 2020*b*), possibly because many sulphur compounds are so reactive and thermally labile (Vazquez-Landaverde *et al.*, 2006; Hougaard *et al.*, 2011). Zabbia *et al.* (2012) also highlighted that Maillard reactions during heat treatment of milk also generate sulphur- and nitrogen-containing compounds. It is likely that any potential differences due to diet may also be exacerbated by thermal-treatments during the production of the WMP.

Only a minority of terpenes or VOC derived from carotenoids were present in these WMP samples. Terpenes, are directly transferred from feed (Kalač, 2011), but also generated through metabolism of sesquiterpenes in rumen or in milk (Fedele *et al.*, 2004). The monoterpenes, α-pinene and 3-carene were significantly more abundant in WMP derived from CLV diets, and D-limonene was significantly more abundant in WMP derived from TMR. Previous studies have highlighted changes in terpene content due to diet and season (Prache *et al.*, 2005; Engel *et al.*, 2007). o-Xylene is also potentially derived from β-carotene degradation or possibly from direct transfer from diet (Kilcawley *et al.*, 2018). In this study, o-xylene was significantly higher in WMP derived from TMR diets. Ethylbenzene, another product of carotenoid degradation (Zhang *et al.*, 2016) was significantly higher ($P < 0.01$) in WMP derived from a CLV diet, but a previous study did not show any impact of diet on ethylbenzene levels in milk (Coppa *et al.*, 2011).

WMP derived from CLV had significantly more acetic acid compared to WMP from either GRS or TMR diets. Acetic acid is primarily a product of carbohydrate metabolism or can be produced from the metabolism of amino acids, but is also known to be directly transferred from feed (Kilcawley, 2017; Clarke *et al.*, 2020*b*). Most studies have found acetic acid in milk, but the dietary impact varies (Croissant *et al.*, 2007; Villeneuve *et al.*, 2013). Diacetyl was significantly more abundant in WMP derived from GRS diets. Diacetyl is widely found in milk and is a result of pyruvate metabolism or direct transfer (Moio *et al.*, 1996).

In terms of consumer acceptance testing, creaminess, aftertaste, cooked-flavour, quality and flavour were significantly different in these WMP based on diet. WMP from CLV was significantly higher for quality, which is probably related to the fact that it was also highest for creaminess and flavour, but significantly lowest for cooked flavour, and aftertaste. Diet is known to influence the composition and distribution of fat and protein in milk, which can impact creamy and smooth perception (Frøst and Janhøj, 2007). O'Callaghan *et al.* (2016) found that milk derived from a CLV diet had a higher level of fat and protein than milk derived from a TMR diet. In this study, WMP derived from CLV diets scored significantly higher for creaminess, which is probably related to the impact of diet on fat content and distribution. Clarke *et al.* (2020*b*) also found similar results where a trained descriptive panel scored creaminess significantly higher in pasteurised milk derived from CLV diets in comparison to milk derived from GRS and TMR diets. It is also plausible that the very abundant lactones (δ-decalactone, δ-dodecalactone and γ-dodecalactone) that differed due to diet also impacted creamy perception (Karagül-Yüceer *et al.*, 2001; Villeneuve *et al.*, 2013). It is difficult to explain a relationship between aftertaste in WMP and the impact of diet, although it may be associated

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with cooked flavour as both were significantly higher ($P < 0.05$) in WMP derived from TMR diets. The development of cooked/milky flavour in UHT milk has been suggested to be derived from milk protein denaturation, particularly serum protein, and possibly also linked to volatile sulphur compounds (Deeth, 2010; Zabbia *et al.*, 2012). However, as mentioned, only dimethyl sulphide was found to be significantly different ($P < 0.01$) in this study based on diet, but at greatest abundance in WMP derived from TMR.

The ODP assessors found a significant impact of cow diet (CLV, GRS and TMR) on WMP for colour, caramelised flavour, sweet aroma, sweet taste, creamy aroma, cooked aroma and cooked flavour. WMP derived from CLV and GRS diets was significantly higher for liking of colour compared to WMP derived from TMR diets (Table 3). This result is similar to that reported by Faulkner *et al.* (2018) for pasteurised milk, and due to higher β-carotene contents. The correlation of the colour of dairy products and β-carotene content has been extensively highlighted and reviewed by Martin *et al.* (2005). ODP data also clearly identified that both sweet aroma, and sweet taste scored significantly higher for WMP derived from TMR diets. This result is similar to that obtained by Villeneuve *et al.* (2013), who found sweet flavour higher in milk from cows fed hay as opposed to pasture and silage, as did Croissant *et al.* (2007), when comparing milk from Jersey and Holstein cows fed TMR *vs.* pasture. Villeneuve *et al.* (2013) speculated that this may be due to the abundance of γ-lactones, which corresponds well with this study where γ-dodecalactone was present at significantly higher abundances in WMP from TMR and is characterised with a sweet, green odour (Karagül-Yüceer *et al.*, 2001). Carmelised flavour was also significantly higher in WMP derived from TMR diets and may also be linked to Maillard reactions (Kilcawley *et al.*, 2018), and/ or to the greater abundance of γ-dodecalactone. Both cooked aroma and cooked flavour were significantly higher in WMP derived from a GRS diet compared to WMP from TMR and CLV diets. This is different to that found in the consumer acceptance part of this study, and in previous studies (Clarke *et al.*, 2020*b*; Manzocchi *et al.*, 2021). The only VOC significantly higher in just GRS WMP were butanal and methyl hexanoate neither of which are associated with cooked flavour. The significant difference in creamy aroma which was higher in WMP derived from CLV than from TMR and GRS diets, similar to that found in the consumer part of this study. As mentioned, this may relate to differences in fat particle-size distribution, fat globule flocculation or fat coalescence due to the presence of lower-melting-point fatty acids and lactones due to diet (Richardson *et al.*, 1993; Villeneuve *et al.*, 2013; O'Callaghan *et al.*, 2016; Faulkner *et al.*, 2018). However, previous studies have not found that diet impacted creamy perception in milk (Manzocchi *et al.*, 2021).

The WMP derived from GRS diets was positively correlated to cooked aroma and with butanal, 2-pentanone, decanal, undecanal, 2-nonanone, γ-crotonlactone, methyl hexanoate and 2-heptadecanone. It is difficult to associate these individual VOCs with cooked aroma as none have previously been directly associated with this attribute. The WMP derived from CLV diets was positively correlated with creamy aroma, dimethyl disulphide, 2-undecanone, ethyl benzene, 2-ethyl-1-hexanol, 1-pentanol, α-pinene, 3-carene and 3-methyl-butanal. None of these VOCs would typically be associated with cream attributes. However, WMP derived from both GRS and CLV were also associated with many δ lactones that are probably influencing the creamy aroma, especially as some were very abundant. The WMP derived from TMR diets was positively associated with carmelised flavour, sweet aroma and oxidised flavour, but also with dimethyl sulphide, *o*-xylene, *p*-xylene, hexanal, 1-hexanol, γ-dodecalactone, D-limonene, heptanal and dodecanal (obtained from the jack-knife uncertainty test, Fig. 3). Sweet taste has previously been found to be a dominant sensory descriptor for WMP derived from TMR diets (Clarke *et al.*, 2021) and sweet has also been associated with concentrate diets, like TMR and γ-lactones (Villeneuve *et al.*, 2013). Chen *et al.* (2017) also found that greater abundances of γ-lactones corresponded with higher scores for the sweet, vanilla and caramel descriptors.

In conclusion, significant differences were evident in the sensory perception and volatile profile of the WMP based on the diet. Consumers rated the overall acceptability, flavour, creaminess and quality of the WMP derived from CLV higher than WMP derived from GRS or TMR diets. Consumers also found that WMP derived from TMR diets scored higher for cooked flavour and aftertaste. The familiarity of the consumers in this study with pasture derived dairy products maybe a factor influencing these results. ODP analysis with trained assessors found that colour was one of the most discriminatory sensory attributes for these WMP based on diet and was significantly positively correlated to WMP derived from CLV diets. Overall ODP analysis found that WMP from all three diets differed, but that WMP from both pasture diets (CLV and GRS) were more closely related, which was confirmed by VOC analysis. The different VOC extraction techniques used provided a more comprehensive volatile profile of these WMP samples, with sixty four individual VOCs identified. Similar trends were evident for those VOCs which were extracted by each technique in relation to the impact of diet. However, DI-HiSorb extracted significantly more VOCs than TD or HS-SPME, but was especially beneficial for extracting lactones. This proved to be very useful as the abundance of some δ-lactones (δ-decalactone and δ-dodecalactone) and γ-dodecalactone were very high. Both δ-decalactone and δ-dodecalactone were significantly more abundant in WMP derived from CLV and GRS diets, with the abundance of γ-dodecalactone significantly higher in WMP from TMR diets. γ-Dodecalactone was probably influencing the sweet aroma and flavour and possibly carmelised flavour associated with WMP from TMR, with δ-decalactone and δ-dodecalactone probably influencing the positive association of creamy aroma with the WMP from the CLV diet. ODP analysis did not find any negative sensory attributes associated with lipid oxidation in these samples, and this is probably due to the fact that they were analysed not long after manufacture, therefore, potential differences did not have sufficient time to develop. It was also apparent that by far most of the significant differences in relation to VOCs in these samples in relation to diet were those primarily derived from fatty acids; lactones, primary and secondary aldehydes, alcohols, ketones and an ester. In hindsight this is not surprising as diet has such a significant impact on the fatty acid profile of milk and is, therefore, likely to impact on subsequent VOCs from this source. This study has also highlighted that differences in VOC due to diet are probably exacerbated by thermal treatment and thus another important contributory factor in WMP manufacture.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029922000589.

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