University of Reading

Department of Food and Nutritional Sciences

Elucidation of the role of different sources of phospholipids in meat aroma formation

HuiQi Yeo

Thesis submitted for the degree of Doctor of Philosophy

Supervisors: Dr Jane K. Parker
Dr Dimitrios P. Balagiannis
Dr Jean H. Koek

October 2022

Declaration

I confirm that this thesis is my own work and the use of all material from other sources has been properly and fully acknowledged. The views expressed in this thesis are those of the author and do not necessarily reflect the views or policies of Unilever or its affiliates.

HuiQi Yeo
6th October 2022

The more I learn,
the more I realise how much I do not know.

– Albert Einstein

Acknowledgments

To my supervisors, Dr J. K. Parker, Dr D. B. Balagiannis and Dr J. H. Koek, it was a privilege to be on board the ride with you. I could not have been more fortunate to have hands-on supervisors who imparted me invaluable lab skills and knowledge, in addition to the support, patience and guidance, especially as we navigated through the uncertainty and complications of the Covid pandemic.

To my past and present colleagues in the Flavour Group, I am deeply blessed for the camaraderie we share and may our paths cross again someday. Sitting at the UoR dinner table that fateful night during the 6th Nursten symposium in Belfast was one of the best decisions I have made in life. To the GC guru in the lab, Dr J. S. Elmore, I truly appreciate the advice and assistance you have provided me during my time here and could only wish that I have learnt more from you. I am immensely grateful for the enriching experience I had within the Flavour Group.

To the technicians in the Department of Food and Nutritional Sciences, Dr O. Khutoryanskaya, Mr A. Whittam, Mr G. Yeo, Mr C. Bussey, Mrs S. Mouring, I am thankful for all the help to overcome the constant logistical challenges, unexpected equipment failures, sporadic electricity cuts and incessant fume cupboard shutdowns during the project. I would also like to acknowledge the assistance of Miss M. Lim for the assistance with the fatty acid analysis and kinetics experiments.

To my first mentor in life, Dr M. Cheong, I am indebted to you for the opportunity to embark on my flavour journey and all the advice you have offered up until today. I am grateful that you continue to keep a lookout for me and glad that we maintained our friendship from miles apart. To my family, thank you for being my *v*ery *p*illar of support. Your incredible patience and tolerance, selfless love and care, had made the difficult times much more bearable, as well as kept my sanity and health in check. To my lab plumber, electrician, handyman, dishwasher, thank you for lending an extra pair of helping hands for these mundane yet necessary tasks. You know you mean more than just that.

To my examiners, Dr L. Farmer and Dr L. Methven, thank you for taking time out of your busy schedules to evaluate my work. It was an immense pleasure discussing my project with you during the viva and I appreciate the feedback provided.

Last but not least, I acknowledge the financial support of Unilever and the Graduate School for the PhD. This was a meaningful project and working with a purpose-driven brand had helped to chart my direction in the food industry.

Thesis organisation

This thesis is composed of an overall abstract, a general introduction (Chapter 1), three individual papers (Chapters 2 – 4), as well as a general discussion and conclusions (Chapter 5). Chapter 1 presents a literature review with a specific focus on the role of phospholipids in meat aroma generation. Chapter 2 investigates the key volatile compounds in boiled chicken (BC) and boiled beef (BB). Chapter 3 examines the role of triglycerides and phospholipids in BC aroma generation. Chapter 4 explores the use of sunflower and soy lipids in the production of BC aroma. Finally, Chapter 5 provides a general discussion and summarised conclusions of the three preceding chapters, along with suggestions for future work.

Thesis abstract

The characteristic aroma of cooked meat is generated from thermally induced reactions, principally the Maillard reaction and lipid oxidation, between non-volatile components of lean and adipose tissues during heating. Although meat aroma formation is a well-documented topic, the shift in consumer landscape towards vegan, vegetarian and flexitarian diets has presented an unprecedented challenge of creating meat aroma from plant-based alternatives. The search for potential vegan solutions has thus led to a renewed interest in authentic meat flavour profiles.

Using Likens-Nickerson simultaneous distillation-extraction, aroma extracts from boiled beef and chicken were obtained for Gas Chromatography-Mass Spectrometry and Gas Chromatograhy-Olfactometry analyses. This technique offers the advantage of in-situ heating and extraction, which facilitates the mimicking of meat cooking in stocks and stews in a kitchen setting. Besides the Maillard reaction products, such as sulfides, thiols and mercaptoaldehydes, as well as lipid oxidation products, such as alkenals and alkadienals, families of thiazoles and thiazolines were also found to be important contributors to meat aroma.

The identification of these odour-active compounds facilitated the investigation of the role of lipids in meat aroma formation. Chicken triglycerides and/or phospholipids were added individually or as a mixture (1:1 w/w) to a defatted chicken matrix and the aroma generated during heating was extracted as before. The results showed that the incorporation of chicken phospholipids significantly enhanced the production of some sulfur- and/or nitrogen-containing Maillard reaction products and most of the lipid-derived products. This was further emphasised in a kinetics experiment, which monitored the accumulation of lipid-derived volatiles over an extended heating duration. The experiment not only demonstrated the higher

efficiency of phospholipids in generating lipid-derived products, but also their catalytic effect on the formation of key volatiles in systems containing triglycerides.

With the aim of finding a suitable plant phospholipid for use in chicken aroma generation, sunflower and soy phospholipids were explored as plausible alternatives in comparison to the triglyceride counterparts using a defatted chicken matrix. Despite the higher saturated and polyunsaturated fatty acid content in chicken and plant lipids respectively, sunflower phospholipids are proposed to be a better choice as compared to the other lipids studied based on the volatile profile of the samples. This also suggests that fatty acid composition is not the only factor to consider in the substitution of animal lipids with plant lipids.

In conclusion, not only are phospholipids more reactive than triglycerides in part due to their higher polyunsaturated fatty acid composition, but also play a catalytic role in the reactivity of the latter. In addition, sunflower phospholipids have the potential for use in the substitution of chicken lipids in plant-based meat products. These findings would be useful for food manufacturers who are keen on the creation and modification of process flavourings or those in search of animal lipid alternatives for application in plant-based meat products.

Research aim, hypotheses and objectives

The overall aim of this work was to investigate the use of plant lipids in meat aroma generation, specifically chicken, based on an understanding of the role of lipids, in particular phospholipids, in meat aroma formation.

1. Research question: What are the differences between boiled beef (BB) and boiled chicken (BC) aroma?

   Hypothesis #1: Differences between BB and BC aroma profiles lie in the presence of different ratios of key volatiles.

   Objective: Identification of key aroma volatiles in BB and BC by GC-O and GC-MS

2. Research question: How do triglycerides and phospholipids affect BC aroma generation?

   Hypothesis #2a: Phospholipids are more reactive than triglycerides due to their higher concentration of unsaturated fatty acids.

   Hypothesis #2b: Phospholipids can catalyse the reactivity of triglycerides.

   Objective: Comparison of the effect of triglycerides and phospholipids on volatile generation and its rate of formation in BC aroma

3. Research question: Can sunflower and soy triglycerides and phospholipids be used to replace chicken lipids in BC aroma generation?

   Hypothesis: #3: Plant phospholipids could be used as a substitution for chicken phospholipids as they both have a high unsaturated fatty acid content.

   Objective: Evaluation of the use of sunflower and soy phospholipids in BC aroma generation in comparison with the respective triglycerides

Contents

1. Role of phospholipids in meat aroma generation

Abstract

Although lipid oxidation is commonly associated with flavour deterioration, it is also an important reaction which occurs during thermal processes such as cooking. A delicate balance of lipid-derived volatiles and Maillard reaction products contributes to the desirable yet complex aroma of cooked meat. Between the two major classes of lipids, triglycerides and phospholipids, it is well-established that the latter are more responsible for the warmed-over flavour in meat but their impact on meat aroma generation has not been closely examined. This review provides a contextual background of phospholipids and aroma generation, as well as a discussion of the available and relevant literature on the pro- and anti-oxidative properties of phospholipids, with a view of their effect on aroma generation. An understanding of the role of phospholipids in meat aroma generation will be useful for the innovation of process flavourings in the food industry, especially since the plant-based market trend demands the need for manufacturers to reassess their raw material portfolio.

Keywords: Phospholipid, prooxidative, antioxidative, lipid oxidation, meat aroma

1.1 Introduction

The shift in consumer landscape towards vegan, vegetarian and flexitarian diets, driven by mounting environmental, animal welfare and health concerns over industrial animal agriculture (He et al., 2020), has led to an explosive growth in the plant-based meat category over the last decade and renewed interest in authentic meat flavour profiles. However, creating meat aroma from plant-based alternatives is challenging and the accurate simulation and manipulation of meat aroma in industrial applications continues to be an ongoing endeavour.

The knowledge of meat aroma has vastly expanded over the past decades and is well documented in reviews ranging from the overarching topic of flavour formation (Mottram, 1998), to aspects of it such as the Maillard reaction (Martins et al., 2000; Rizzi, 2008; van Boekel, 2001, 2006) and lipid oxidation (Amaral et al., 2018; Dominguez et al., 2019; Ladikos & Lougovois, 1990; Min & Ahn, 2005), as well as those with a focus on specific animal species like chicken (Jayasena et al., 2013) and beef (Kerth & Miller, 2015). Many patents have also been filed for the innovative preparation of meat flavourings and examples of those which involve the use of lipids describe the addition of vegetable fats in the presence of an oxidant (Eshuis, 2011), phospholipid with a reactive S-containing compound (Lee & Tandy, 1991) or sal butter (obtained from the *Shorea robusta* tree native to India) in meat-like food products (Timmons et al., 2021)

Among the plethora of literature on meat aroma, little emphasis had been given to discuss the impact of phospholipids on the generation of desirable meat aroma. It is worthy to note that warmed-over flavour, which was first coined by Tims and Watts (1958), describes the rapid onset of rancidity in cooked meat during short-term

refrigerated storage and is different from the pleasant characteristics of meat aroma. While there has been extensive research on the influence of phospholipids on the development of warmed-over flavour, relatively little has been reported on its role in meat aroma generation, which also deserves attention since phospholipids participate in lipid oxidation and thus, are important contributors of meat aroma. Due to the limited number of studies investigating phospholipids in meat matrices, oil-in-water (O/W) emulsions will also be considered in this review given that the meat matrix can be classified as an emulsion made up of fat globules dispersed in a continuous aqueous solution containing salt-soluble myofibrillar proteins and other components such as insoluble proteins, muscle fibres and connective tissues (Mandigo & Sullivan, 2014). Moreover, in cooking situations such as the boiling of soups, stocks and casseroles, the meat is usually suspended in water and the lipids could be released from the matrix into the aqueous environment under thermal conditions. Phospholipid oxidation is undoubtedly a broad topic and thus, for further insights on the influence of phospholipids on lipid oxidation in other food systems, as well as the in-depth chemistry behind it, one is referred to the respective reviews by Cui and Decker (2016), as well as Min and Ahn (2005) and Reis and Spickett (2012).

In this review, the essential knowledge of phospholipids and meat aroma generation is summarised. This provides contextual background for the discussion of the available literature about the influence of phospholipids on lipid oxidation in meat and meat-related matrices, offering perspectives of phospholipids as pro- and anti-oxidants and the consequence on meat aroma generation. Finally, recommendations for future research are proposed.

1.2 Phospholipid classification, structure, function and sources

1.2.1 Chemical classification

The primary classification of phospholipids is based on the backbone structure and phosphate head group. Glycerophospholipids consist of a glycerol backbone with an alkyl or alkenyl, or acyl chain attached at the *sn*-1 position via an ether or ester linkage respectively, a fatty acid esterified at the *sn*-2 position and a highly polar or charged group attached at the *sn*-3 position via a phosphodiester linkage. The simplest glycerophospholipid is phosphatidic acid (PA) with a free phosphate group while the attachment of other chemical groups gives rise to the major classes such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) (Ruiz et al., 2009). Plasmalogens are also a class of glycerophospholipids with a fatty alcohol linked at the *sn*-1 via a vinyl-ether bond (Wu et al., 2021). Meanwhile, phosphosphingolipids are composed of a sphingoid backbone with a single fatty acid and a polar head group attached via an amide and phosphodiester linkage respectively (Fahy et al., 2005). Sphingomyelin (SM), a member of this family, is the major sphingolipid found in animals but not found in higher plants (Tafesse et al., 2006). Figure 1-1 shows the structures of these major classes of phospholipids. The secondary classification is according to the *sn*-1 and *sn*-2 substituents, such as fatty acids with variable carbon chain length, as well as number and position of double bonds. Thus, molecular profiles of phospholipids are highly complex and diverse.

1.2.2 Physical structure and function

The presence of a hydrophilic polar phosphate head group and hydrophobic hydrocarbon chains attached to a glycerol backbone confer on the phospholipids their

amphiphilic character, which promotes the formation of a variety of macromolecular structures in the presence of water. The classical representation of phospholipid organisation is a bilayer, whereby the polar regions orient toward the aqueous phase while the hydrophobic regions are sequestered from water in a 1-dimensional fluid lamellar phase (L_{α}). Another common structure is a 2-dimensional inverse hexagonal phase (H_{II}), which consists of fluid hydrocarbon chains penetrated by hexagonally packed aqueous cylinders (Erickson, 2008; Seddon & Cevc, 1993). An illustration of these structures is provided in Figure 1-2.

X-ray diffraction techniques (Luzzati & Tardieu, 1974), vibrational spectroscopies such as Fourier transform infrared (FT-IR) spectroscopy (Mantsch & McElhaney, 1991), as well as nuclear magnetic resonance (Akutsu, 2020) can be employed for such structural elucidation. However, phospholipids do not always adopt a single conformation but instead display polymorphism. PE has been shown to exhibit bilayer-hexagonal phase transition, which can be influenced by temperature, pH and ionic strength (Hardman, 1982; Harlos & Eibl, 1980; Seddon et al., 1984). Other molecular assemblies which are less commonly adopted by phospholipids are discussed in the review by Luzzati and Tardieu (1974).

Phospholipids are ubiquitous to all living organisms and are essential components of cell membranes. A primary function of phospholipid bilayers is to serve as selectively permeable barriers of cells and organelles, which define their separation from the surroundings and regulate the transport of cellular constituents such as nutrients and ions (Lordan et al., 2017). In addition, phospholipids also provide the matrix for the assembly and function of a wide variety of catalytic processes by acting as precursors in the synthesis of macromolecules and by actively influencing the functional properties

of membrane-associated processes. An example is the ability of phospholipids, to facilitate the formation of organisational sites within the membrane for the embedment of multi-unit complexes comprised of both membrane-integral and cytoplasmic proteins, which are involved in important cellular functions such as signal transduction, solute transport and cell recognition, within the phospholipid bilayer (Dowhan, 1997). However, it is not only the bilayer structure which is of biological significance and the importance of phospholipid polymorphism has been pointed out by Dowhan (1997) in his review that the collective physical properties of membranes significantly influence diverse processes such as conformational changes in enzymes and cell division. These non-bilayer structures may play dynamic roles in membrane mediated processes such as membrane fusion and lipid redistribution across the bilayer via transitory formation of intrabilayer inverted lipid structures (Cullis & De Kruijff, 1979).

1.2.3 Sources

In the food industry, phospholipids are more widely known as lecithins, which are defined in the Food Chemical Codex as a naturally occurring mixture of the phosphatides of choline, ethanolamine, and inositol, with smaller amounts of other lipids. The plant sources are commonly derived from the oilseeds of soya, rapeseed and sunflower while animal sources usually originate from milk, egg yolk and brain matter. Marine sources of lecithin may also prove to have applications in the future due to their potential nutritional benefits (Lu et al., 2017).

Lecithin production on an industrial scale varies depending on the raw material. The primary method of plant lecithin production is through an operation in edible oil refining and purification known as degumming. During this process, crude vegetable oil is heated at 50 – 70 °C and steam or water (2 – 3%) is added with slow agitation

to hydrate the polar lipids, making them insoluble. The hydrated gums are removed by continuous centrifugation before drying by film evaporation and cooled to obtain crude lecithin (Szuhaj, 1983; van Nieuwenhuyzen, 1976). Unlike plant lecithins which are obtained as by-products during the purification of triglycerides, animal lecithins are produced in a fundamentally different way. In egg yolk, the tight binding of phospholipids to proteins requires the use of alcohols for bond disruption (Schneider, 1989) and thus, an acetone-ethanol mixture has long been used for the extraction of crude lecithin from liquid egg yolk (Hanahan et al., 1951). Spray- or freeze-dried egg yolks could also be used as starting materials in solvent-free filtration techniques as claimed in a patent filed by Hatanaka Hiroji (1991).

Physical, chemical and enzymatic modifications can be made to crude lecithins to achieve standardised products with desired quality parameters, such as acetone insolubles, acid value, colour and phospholipid composition (Szuhaj, 1983), or structurally diverse products which are optimised for a range of applications (Doig & Diks, 2003; Joshi et al., 2006; van Nieuwenhuyzen, 1981). For instance, blending of different crude lecithins is common practice to attain a final product of consistent composition and functionality (Bueschelberger et al., 2015). The phospholipid content of some commercial lecithins is listed in Table 1-1. However, the exact composition of lecithin depends on the source and quality of the oilseeds, as well as the degumming conditions. Beyond lecithins, a compilation of the phospholipid content in meats and other foods was provided by Weihrauch and Son (1983).

1.3 Meat aroma generation

The characteristic aroma of cooked meat is generated from thermally induced reactions, principally the Maillard reaction and lipid oxidation, between non-volatile

components of lean and adipose tissues during heating. These complex reaction pathways lead to the formation of a vast range of products, accounting for the diverse classes of volatile compounds found in cooked meat as presented in Figure 1-3 (Mottram, 1998). While the aroma compounds produced during the Maillard reaction are typically responsible for the savoury, meaty, roast and boiled characters, those derived from lipid oxidation impart fatty aromas to cooked meat and can contribute to species specific notes (Macy et al., 1964).

1.3.1 Maillard reaction

The Maillard reaction is the basis for flavour formation in a wide range of thermally processed foods and not only occurs in savoury foods such as meat and vegetables like potatoes, but also in sweet goods and beverages such as bread, chocolate, tea and coffee (Cerny, 2008). This complex cascade of parallel reactions can be divided into three stages. The initial stage starts with a condensation between a reducing sugar and an amino compound to form a Schiff base, which rearranges into an Amadori or Heyns Rearrangement Product (ARP or HRP) in the case of an aldose or ketose respectively (Hodge, 1953). During the intermediate stage, the fragmentation of the ARP regenerates the amino acid and produces 1-deoxyhexosulose (1-DH) and 3-deoxyhexosulose (3-DH) with the formation of the latter favoured at lower pH (Tressl et al., 1995). The formed diketone structure of deoxyhexosuloses, also known as glucosones, are very reactive and can subsequently undergo structural rearrangements or chemical reactions to create a pool of dicarbonyl and hydroxycarbonyl compounds, some of which are odour active. The retroaldolisation of 1-DH and 3-DH produces pyruvaldehyde and glyceraldehyde. Furthermore, the rearrangement and dehydration of 1-DH give rise to diacetylformoin, which is the precursor for the formation of other reactive intermediates such as acetaldehyde, 2,3-

butanedione, and hydroxypropanone. As these intermediates are highly reactive, they participate in an array of reactions, such as addition reactions with hydrogen sulfide and ammonia, aldol condensations, dehydration and cyclisation reactions, to generate a range of other aroma compounds (Parker, 2017). Non-volatile compounds such as acetic and formic acids could also be produced from 1-DH and 3-DH respectively (Brands & van Boekel, 2001). The Strecker degradation is another important reaction for the creation of potent aroma compounds as it provides routes by which nitrogen and sulfur can be introduced into heterocyclic ring systems during the Maillard reaction. It involves a carbonyl-amine condensation usually between an α-dicarbonyl compound (e.g. deoxyosones) and an amino acid to form the corresponding α-aminoketone derived from the sugar moiety, and Strecker aldehyde derived from the parent amino acid but containing one carbon atom less, which is released as carbon dioxide (Hodge, 1953).

In meat, the water-soluble compounds such as free sugars, amino acids and nucleotides, which exist naturally or develop post-slaughter, serve as the precursors for the Maillard reaction (Macy et al., 1964). Ribose, an important pentose sugar present in the muscle, is released during the cascade of enzymatic reactions involved in the post-mortem breakdown of adenosine triphosphate, which is an essential ribonucleotide for muscle function (Lawrie & Ledward, 2006). As for the amino acids, the S-containing cysteine and methionine are the ones which contribute to the generation of savoury, meaty aromas. The Strecker degradation of cysteine yields mercaptoacetaldehyde and several different reactive intermediates (Fujimaki et al., 1969). Hydrogen sulfide and ammonia can react with the pool of reactive carbonyl compounds generated from the Maillard reaction for the formation of heterocyclic aroma compounds such as pyrazines, thiophenes and thiazoles (Cerny & Davidek, 2003;

Farmer et al., 1989; Mottram, 2007; Zhang & Ho, 1991). In addition, the interaction of hydrogen sulfide with dicarbonyls, furanones and furfurals produces thiols and mercaptoketones, which can form a mixture of disulfides upon oxidation (Mottram, 1998).

Meanwhile, the Strecker degradation of methionine produces methional (Ballance, 1961; Fujimaki et al., 1969), which imparts a distinct baked or boiled potato aroma often detected in chicken and beef (Gasser & Grosch, 1990). Being unstable, this Strecker aldehyde can subsequently break down to form methanethiol (Yu & Ho, 1995), which readily oxidises to dimethyl disulfide (Pripis-Nicolau et al., 2000), as well as provide the 2-methylfuranyl moieties in compounds, such as 2-methyl-3-furanmethylsulfide. The Strecker degradation pathways of these amino acids are shown in Figure 1-4.

The production of N-containing compounds can also arise from the reaction between Strecker degradation products. Pyrazines can be formed from the carbonyl-amine condensation between α-aminoketones and their subsequent oxidation (Shibamoto & Bernhard, 1977). For the more substituted pyrazines, the methyl and ethyl substituent groups can be derived from formaldehyde and acetaldehyde respectively, and these short-chain aldehydes are the degradation products of glycine and alanine respectively (Amrani-Hemaimi et al., 1995). Some of the other classes of compounds derived from the Maillard reaction are shown in Figure 1-3.

In the final stage of the classic Hodge scheme, the majority of the reactions involve polymerisation and colour-forming pathways as the focus is on Maillard browning. Nevertheless, the formation of heterocyclic compounds from the rich pool of reactive

intermediates derived from the earlier stages is of paramount importance for aroma generation. Besides the nature and availability of the precursors, the various possible reaction routes which occur are strongly dependent on temperature, pH and moisture of the system (van Boekel, 2006).

1.3.2 Lipid oxidation

The lipid autoxidation process is a free radical-induced chain reaction which occurs in three stages: initiation, propagation, and termination. The initiation stage is triggered by the abstraction of a hydrogen atom from the lipid by a free radical species, resulting in the formation of a lipid radical, which reacts with molecular oxygen to form a lipid hydroperoxide during the propagation stage. As the abstraction of an allylic hydrogen atom, which is located next to an unsaturated double bond of a fatty acid, occurs readily due to the formation of resonance-stabilised lipid radicals (Jansen, 2014), unsaturated fatty acids are more prone to oxidation, particularly at low temperatures. On the other hand, saturated fatty acids (SFAs) are relatively more stable and their degradation becomes more important at elevated temperatures (Parker, 2014).

Lipid hydroperoxides break down by the cleavage of the C–C bond on either side of the radical. With SFAs, the lack of a double bond to guide the initial abstraction of hydrogen leads to the formation of a series of alkanes, alkanals, and alcohols with different chain lengths. On the other hand, lipid degradation is more directed in unsaturated fatty acids, with polyunsaturated fatty acids (PUFAs) being more reactive than monounsaturated fatty acids (MUFAs) due to the higher number of possibilities for hydrogen abstraction. Nevertheless, the dominant pathways are dependent on the relative stability of the lipid radicals and the compounds that are formed, as well as reaction conditions.

In meat, muscle lipids are composed of neutral lipids, which are primarily comprised of triglycerides in the adipocytes located along the muscle fibres and in the interfascicular area, as well as polar lipids, which mainly consist of structural phospholipids located in the cell membranes (Raes & De Smet, 2009). As depicted in Figure 1-1, the structure of triglycerides is relatively simple compared to other classes of lipids, consisting of 3 fatty acids each attached to a glycerol backbone via an ester linkage. On the other hand, the structural complexity of phospholipids is manifested in the variety of chemical groups attached to a backbone comprised of either a glycerol, which is trihydroxy sugar alcohol, or a sphingosine, which is a long chain amino alcohol (Lehninger et al., 2012).

Besides structural differences, the two classes of lipids also exhibit variations in their fatty acid composition. While triglycerides have a higher proportion of SFAs and MUFAs, phospholipids are rich in PUFAs (Raes & De Smet, 2009), which render them more susceptible to oxidation, especially when they are released from the cell membranes into the aqueous environment where they come into contact with potential catalysts, such as reactive oxygen species and iron present as cations, in cytochromes or haemoglobin (Erickson, 2008). However, this also confers phospholipids a greater role in aroma development than triglycerides (Mottram & Edwards, 1983).

1.4 Properties of phospholipids and effect on meat aroma generation

1.4.1 Structure and stability

Besides the fatty acid composition of the phospholipids, in particular the number and location of the double bonds (Miyashita et al., 1995), the structural diversity in polar head groups can also confer distinct characteristics on the lipid molecule and influence

the type of chemical reactions that occur. Under different pH conditions, the protonation or deprotonation of these functional groups changes the chemical nature and net charge of the phospholipid, which in turn affect their molecular conformation and interaction with ionic species in the environment. While the phosphate groups (PO_4^-) have a pKa ~ 2, the primary amino groups (NH_2) on PE and PS have a pKa ~ 9.5 and the carboxyl group (COOH) on PS has pKa ~ 3 (Tatulian, 1993). At the pH of meat (pH 5.5 – 6), the phosphate and carboxyl groups are deprotonated, while the amino group remains protonated. Thus, PS carries a net negative charge of -1 while PE exists as a zwitterion with a net zero charge. As the quarternary amino group of PC can become protonated within this pH range, and coupled with the negative charge of the phosphate group, PC can also exist as a zwitterion.

In addition, the assortment of molecular assemblies, such as bilayers, micelles or vesicles, formed by phospholipids when dispersed in water, possess unique interfacial properties which can have an impact on emulsion stability (Pichot et al., 2013). The characteristics of the interfacial area, such as electrical charge, thickness and packing density, are of paramount importance as they affect the chemical interactions between the phospholipids and the aqueous phase constituents, such as prooxidants and antioxidants (Berton-Carabin et al., 2014). The intricate relationship between physical and chemical emulsion stabilities is highlighted in the study whereby emulsions prepared using commercial sunflower lecithin with lower PC level were more physically stable due to their smaller droplet sizes and were therefore more chemically stable than those composed of lecithin with higher PC level as measured by lipid hydroperoxide and TBARS concentrations (Liang et al., 2017).

1.4.2 Prooxidative v.s. antioxidative properties

Theoretically, the adsorption of phospholipids at the interface of a lipid droplet in an O/W emulsion could build up electrical charges with a sign and magnitude determined by the type and concentration of the charged surface-active species in the molecular environment (Mcclements & Decker, 2000). Thus, the negative charges present on the phosphate head group could lead to the electrostatic attraction of metal cations, especially those with higher valency (e.g., Fe^{2+}), thereby promoting lipid oxidation as a result of the close proximity between the fatty acids and prooxidants, as observed in O/W emulsions stabilised by sodium dodecyl sulfate, which is an anionic surfactant (Mancuso et al., 1999; Mei et al., 1998). This was also cited as the prooxidant mechanism of free fatty acids in stripped soybean O/W emulsion (Waraho et al., 2009). Moreover, the amphiphilic nature of phospholipids facilitates the decrease in interfacial tension between two immiscible phases, thereby contributing to their excellent emulsifying capacity and water dispersibility (Cui & Decker, 2016). This resultant formation of a high interfacial area favours the accessibility of the lipid vesicles to hydrophilic prooxidants dissolved in the aqueous phase (Berton-Carabin et al., 2014), which in turn promotes lipid oxidation. Chen et al. (2019) also proposed that the reduction in surface tension could enhance the rate of oxygen diffusion from the lipid droplet surface to the interior, resulting in the acceleration of lipid oxidation within the oil matrix.

On the other hand, several mechanisms have been proposed to contribute towards the antioxidative properties of phospholipids. A tight intermolecular packing arrangement of phospholipids was speculated to protect the constituent PUFAs from oxidation, as observed in the higher oxidative stability of salmon egg PC than soybean PC in a Tween 20-stabilised phosphate buffer (pH 7.4) despite the greater number of

bisallylic positions in the presence of more highly unsaturated PUFAs (eicosapentaenoic acid, or EPA, and docosahexaenoic acid, or DHA) (Miyashita et al., 1994). This theory was based on the computer modelling of DHA which indicated that the PUFA could promote tight chain packing arrays in aqueous medium (Applegate & Glomset, 1986), as well as the selective terminal oxidation of n-3 FAs in aqueous glyme (Kato et al., 1992). However, the computer scientists also concluded their findings with the caveat that a SFA and DHA had to be in the *sn*-1 and *sn*-2 positions respectively and unlikely to be applicable for PC as the large choline head group would be too large to be compatible with the simulated conformation. Nevertheless, the same results were also achieved by the research group using liposome models, in which salmon egg PC was more oxidatively stable than soybean PC. In this later study, it was suggested that the selective distribution of PUFAs at the sn-2 position instead of sn-1 could increase the resistance of salmon egg PC bilayers to oxidation (Nara et al., 1997).

In comparison between different classes of phospholipids, egg PE was reported to be more oxidative than egg PC when dispersed in Tween 20-stabilised borate buffer (pH 7) as indicated by a shorter induction period determined by oxygen uptake (Corliss & Dugan Jr., 1970). The observation was attributed to the difference in nitrogen moiety as there was only a 1% difference in the concentrations of arachidonic acid, which was found to be the more oxidative of the two major PUFAs in the phospholipids present (the other being linoleic acid). This was measured by the amount of each PUFA remaining at various intervals over the period of lipid oxidation. It was interesting that the amount of both PUFAs remaining only took a sharp decline after 10 h in the PC O/W emulsion but no arachidonic acid was found in the PE O/W emulsion after 6 h and only half the initial concentration of linoleic acid was remaining after 8 h. The higher reactivity of PE than PC was also observed in synthesised phospholipids by

measurement of their hydroperoxide formation based on the absorption of conjugated dienes (Kawakatsu et al., 1984). Yin and Faustman (1993) also observed that lipid oxidation increased in the order of PC < PE < PS in oxymyoglobin-phospholipid liposome models, owing to the negative charge of PS and the neutral charges of PC and PE at pH 5.6 as the PUFA composition could not explain the results (higher oxidation found in liposomes with less PUFAs). However, the researchers noted that within the same PL head group, an increase in chain length and fatty acid chain unsaturation resulted in an increase in oxidation, although such an effect on stability was not found in pure PC liposomes (Araseki et al., 2002) and an opposite trend was in fact reported in emulsions (Miyashita et al., 1993; Waraho et al., 2011). It was postulated by the latter groups of researchers that the structural conformation of the fatty acids could affect their rate of oxidation. The linear shape of MUFAs facilitate their access to the emulsion droplet surface as evidenced by their ability to decrease the negative surface charge and thereby, reducing the attraction of positively charged prooxidants and susceptibility to oxidation. On the other hand, PUFAs such as DHA and EPA could adopt a more tightly packed conformation, rendering it difficult for free radicals and/or oxygen to attack the substrates.

Besides the structural conformation of phospholipids, their ability to undergo chemical reactions with other components of the system can also bring about antioxidative effects. In marine phospholipid liposomal dispersions, PE was reported to offer enhanced protection against lipid oxidation due to the formation of antioxidative, hydrophobic pyrroles from reactions between the primary amino group in PE and tertiary lipid oxidation products. Thus, lesser lipid oxidation was observed in PE than PC (Lu et al., 2013). In addition, it was demonstrated that PE and PS could regenerate α-tocopherol through amino-carbonyl reactions but PC lacked the ability to do so due

to its quarternary amino group (Doert et al., 2012), which instead contributed to the reduction in antioxidative capacity of PC upon oxidation (Hidalgo et al., 2005). Meanwhile, the antioxidative effect of PA was attributed to the suppression of the catalytic activity of non-haem iron and hemoprotein (Dacaranhe & Terao, 2001). Thus, it was reported that PE and PS exhibited synergism with tocopherols in the inhibition of lipid oxidation in soybean O/W emulsions (Samdani et al., 2018)

The prooxidative or antioxidative capacity of phospholipids has been shown to be pH dependent. Cardenia et al. (2011) observed that lipid oxidation in 1% stripped soybean O/W emulsion was inhibited by PC at pH 7 but accelerated at pH 3. In addition, the chain length of the fatty acids esterified to the PC also affected the extent of impediment at pH 7, which was suggested to be due to the greater partitioning of dibutyroyl PC into the aqueous phase than dioleoyl PC, resulting in a higher rate of lipid oxidation, presumably due to interactions with prooxidants in the aqueous phase. With experiments carried out under conditions to eliminate or investigate the possibility of antioxidative Maillard reaction product formation, lipid hydroperoxide decomposition and changes in the lipid droplet surface charge (zeta potential), the researchers proposed that the antioxidative mechanism was through metal chelation or physical effect. Such an influence of pH on lipid oxidation was also observed in egg and soy lecithin emulsions (Wang & Wang, 2008), as well as oxymyoglobin-phospholipid liposome models (Yin & Faustman, 1993) but was attributed in the latter study to the increased protonation of bound oxygen and release of prooxidant superoxide anion in a low pH environment (Livingston & Brown, 1981). On the other hand, a decrease in pH could also lead to a higher concentration of positive ions (H^+) near the surface of liposome vesicles, resulting in a reduced attraction of catalytic metal cations to the

surface where oxidation occurs, resulting in a lower rate of lipid oxidation observed in phospholipid liposome dispersions (Mozuraityte et al., 2016).

Not only does pH affect the behaviour of phospholipids, it can also promote changes in the other components present in the system. The activity of prooxidants will particularly have an impact on their interaction with phospholipids and therefore lipid oxidation. For instance, it was demonstrated the autoxidation of ferrous iron (Fe^{2+}) to ferric ion (Fe^{3+}) was enhanced in aqueous systems at pH ≥ 7, resulting in the formation insoluble ferric hydroxide with poor catalytic activity (Kawakatsu et al., 1984). It was proposed that the precipitation of iron on the lipid droplet surface at high pH would bring the catalyst and substrate in closer contact with each other, thereby increasing lipid oxidation (Mancuso et al., 1999). Alternatively, the negative charge on the liposome vesicles at high pH could improve their attraction with Fe^{3+} and this retention would prevent the complete precipitation of Fe^{3+}, thereby facilitating lipid oxidation (Mozuraityte et al., 2016). Both are plausible explanations for the prooxidant effect of phospholipids in the presence of an iron catalyst at high pH. Furthermore, proteins which are a major component of meat matrices and reportedly exhibit an antioxidative effect on phospholipids (Miyashita et al., 1994) could display distinct structural conformations and behaviours under different pH conditions, thereby altering their interactions with the phospholipids.

The discrepancies between these studies could arise from a plethora of factors. To begin with, the sample preparation method was different, including but not limited to the sample composition, as well as homogenisation conditions. Moreover, the reaction conditions employed in the oxidation process were diverse, such as temperature, pH, presence of catalysts such as Fe^{2+} (Kawakatsu et al., 1984; Mancuso et al., 1999;

Mozuraityte et al., 2016) and ascorbic acid (Kawakatsu et al., 1984), as well as emulsifiers such as non-ionic surfactants like Tween 20 (Miyashita et al., 1994) and anionic surfactants like sodium dodecyl sulfate (Mancuso et al., 1999; Mei et al., 1998). Furthermore, the measurement of lipid oxidation was distinct to each study, such as oxygen uptake using a differential respirometer (Corliss & Dugan Jr., 1970), thiobarbituric acid reactive substance (TBARS) concentration (Corliss & Dugan Jr., 1970; Hidalgo et al., 2005; Yin & Faustman, 1993), unsaturated fatty acid concentration (Miyashita et al., 1994), lipid hydroperoxide concentration (Cardenia et al., 2011; Corliss & Dugan Jr., 1970; Lu et al., 2013) and headspace concentrations of lipid oxidation products such as hexanal (Lu et al., 2012; Rizzi, 2008) as indicators of primary and secondary oxidation respectively. A review of the various physical and chemical methods is provided by Gray (1978) and despite its early publication, is still a helpful reference for readers who require basic guidance on the selection of a suitable method. It is generally recognised practice to employ at least two types of tests to monitor the different stages (early and late) of lipid peroxidation and it is also recommended that electron- or radical-scavenging assays be used to complement methods associated with lipid peroxidation (Moon & Shibamoto, 2009).

Hence, oxidation conditions employed in model systems should be relevant to flavour development and/or deterioration in the food matrix of concern and the method adopted to determine the extent of lipid oxidation should be robust. Although the headspace technique used in the measurement of the concentrations of lipid oxidation products is fast gaining popularity today due to its convenience and speed, there could be different retention capacities of volatiles in emulsions with different formulations or matrices and thus, calibration curves matching the matrices should be prepared as in the work of Lu et al. (2012), which would also facilitate comparison between studies.

1.4.3 Effect on meat aroma generation

Several studies examined the impact of phospholipids on warmed-over flavour (Igene & Pearson, 1979; Willemot et al., 1985) and the general consensus is that phospholipids are major contributors to this phenomenon. On the contrary, fewer investigations were carried out to elucidate the role of phospholipids in meat aroma generation in terms of volatiles and odours detected.

The first foray into this research topic was made by Mottram and Edwards (1983) who demonstrated that the removal of trigylcerides from freeze-dried and reconstituted lean beef extracts using petroleum ether had little effect on the aroma of cooked meat when heated at 100 °C for 20 min but the removal of both trigylcerides and phospholipids using chloroform/methanol mixture resulted in the substitution of the meaty aroma by generic roasted notes. This was attributed to an increase in pyrazine formation, such as methylpyrazine, 2,5/6-dimethylpyrazine and trimethylpyrazine, as well as a decrease in aliphatic lipid-derived aldehydes, such as hexanal, heptanal and nonanal, as analysed by dynamic headspace extraction (DHE). Since pyrazines are characteristic Maillard reaction products, it was proposed that lipids or its degradation products acted as inhibitors of the formation of heterocyclic products during the reaction. The study was later emulated in pork samples and the same conclusions were drawn based on headspace solid-phase microextraction (HS-SPME) aroma analysis (Huang et al., 2010).

The effect of phospholipids was also studied in model systems containing sugars and amino acids using Type III-E egg yolk lecithin (PC). In aqueous solutions of ribose with either glycine, cysteine or lysine heated at 140 °C for 1 h and pH 5–6, it was found that phospholipid introduction had a quenching effect on the production of heterocyclic

compounds which were derived solely from the Maillard reaction (Whitfield et al., 1988). This was suggested by the researchers to be due to the competition of precursors, such as hydrogen sulfide and ammonia, for the formation of S-containing compounds (e.g. furanthiols) and N-containing compounds (e.g. alkylpyrazines) respectively by lipid degradation products. Alternatively, the condensation of these compounds with lipid oxidation products, such as fatty aldehydes, to form non-volatile polymeric materials could also lead to the reduction in concentrations of heterocycles. However, a distinct trend was not always observed, as in the case of alkylpyridines, whereby there was an increase in the concentration of some compounds but a decrease in others.

Meanwhile, the introduction of a slightly different variation of lecithin (type IX-E from fresh frozen egg yolk containing up to 30% PE) under the same conditions resulted in the generation of lipid-derived compounds over a wide range of classes, such as saturated and unsaturated aliphatic alcohols, aldehydes and ketones, in glycine-ribose (Salter et al., 1988) and cysteine-ribose systems (Farmer et al., 1989), which were not reported in the previous study from the same laboratory (Whitfield et al., 1988). Gas chromatography–odour (GC–O) assessments revealed that phospholipid addition increased the variety of odours perceived, which corresponded with an increase in the overall number of volatiles detected by gas chromatography–mass spectrometry (GC–MS). In the glycine-ribose system, the inclusion of phospholipids introduced green, aldehydic and fatty odours, which could originate from hexanal, heptanal, octanal and nonanal. However, high levels of lipid-derived aldehydes also masked the odours of other compounds as observed in the loss of mushroom and metallic notes from 1-octen-3-one. As for the cysteine-ribose system, phospholipid addition resulted in an increase in the number of compounds possessing meaty aromas perceived by GC-O,

as well as the formation of lipid-Maillard products such as alkyl- and alkenyl-thiophenes, alkanethiols and pentylpyridine, which were only identified in phospholipid-containing systems.

More varieties of phospholipids, namely purified beef phospholipid (containing PE and PC), as well as egg yolk PC and PE, in addition to beef triglycerides were also explored in cysteine-ribose systems by Farmer and Mottram (1990). Not only did these researchers validated their previous findings that phospholipids led to a more pronounced reduction in heterocycle formation (Farmer & Mottram, 1990; Mottram & Edwards, 1983; Salter et al., 1988; Whitfield et al., 1988), such as mercaptocarbonyls and thiol-substituted compounds reported in this study, as compared to trigylcerides, they also discovered that the addition of egg PE conferred on the system a more pleasant meaty note than that of egg PC, which was ascribed to the differences in their PUFA composition and polar moieties. Various mechanisms of lipid-Maillard interaction were also proposed: a) the reaction of lipid-derived carbonyl products with the amino groups of cysteine and ammonia produced during Strecker degradation; b) the reaction of lipid-derived hydroxy and carbonyl products with free hydrogen sulfide; c) the interaction of free radicals from peroxidised lipids in the Maillard reaction. Such unique effects exerted by different classes of phospholipids were also observed in heated aqueous dispersions of 2% high purity (>99%) egg PC and PE in phosphate buffer at pH 5.6 (Lin & Blank, 2003). It was found that the amount of (E,E)-2,4-decadienal in PC was about 20-fold higher compared to PE while hexanal was the major odour-active compound in PE, which explained the fatty, fried and metallic odour of PC but fishy, green and metallic notes in PE. As the differences in the fatty acid composition of PC and PE could only partially explain the quantitative results found in

this study, the researchers suggested that other parameters might have influenced the formation of carbonyls from heated aqueous dispersions of phospholipids.

These researchers subsequently followed up with another study, which correlated the concentrations of odour-active carbonyl compounds measured in heated aqueous dispersions of 10% phospholipid in phosphate buffer (pH 5.6) with phospholipid conformation based on theoretical phase diagrams (Lin et al., 2004). The more efficient generation of carbonyl odourants, such as 1-octen-3-one, (E)-2-octenal and (E,E)-2,4-decadienal, observed in PC-containing systems as compared to PE, was proposed to be attributed to the lamellar phase adopted by PC, unlike the reverse hexagonal structure of PE, although the mechanisms behind the effect of structural conformation on volatile generation remained unknown. However, one can draw parallel from observations in W/O (monoglyceride) systems. It was reported that structured fluids with cubic phases were more efficient in flavour generation than O/W microemulsions as determined by furfurylthiol and 2-methyl-3-furanthiol concentrations due to 'cubic catalysis', a term the researchers used to describe the acceleration of reactions attributed to interfacial properties which facilitated the partitioning and close proximity of the reactants at the interface (Vauthey et al., 2000). Among the monoglyceride-based mesophases, the hexagonal phase was reported to be more optimum for xylose degradation and thus, norfuraneol accumulation, in a xylose-glycine system as compared to the lamellar and cubic phases (Blank et al., 2006). All these studies had demonstrated that the molecular organisation of the reaction medium could influence the thermal generation of volatiles and play an important role in food systems containing ingredients which could form self-assembly structures.

Although an increase in lipid oxidation products can effectively quench reactive intermediates from the system and interfere with the progress of the Maillard reaction, the production of lipid oxidation products, such as saturated and unsaturated aldehydes, can also contribute to the Maillard reaction by participating in the Strecker degradation of amino acids via analogous mechanisms (Hidalgo & Zamora, 2004). Since common intermediates, such as acetaldehyde, pyruvaldehyde and oxaldehyde, exist in the Maillard reaction and lipid oxidation pathways, both reaction cascades can influence each other through enhancement or suppression effects, and even result in the generation of new compounds. Due to the complex interplay between the Maillard reaction and lipid oxidation pathways during thermal processing, the simultaneous consideration of both reactions is necessary for the understanding of the reaction mechanisms, kinetics and products in the complex mixture of carbohydrates, lipids and proteins present in meat. Unlike the aqueous model systems above, opposite results were achieved when water was eliminated. Instead, the addition of phospholipid to vacuum-dried phosphate buffer extracts of cysteine and ribose only led to a minor effect on the volatile profile, with the formation of small quantities of lipid-derived and lipid-Maillard products, which was attributed to the inhibition of the release of free fatty acids from the phospholipids in the absence of water (Mottram, 1995).

The most recent studies were carried out with the aim of using egg yolk phospholipids to boost or imitate chicken aroma. When 1% w/w egg yolk phospholipids were added to minced chicken breast meat and boiled as a broth at 100 °C for 20 min, it was observed that there was a significant increase in most of the lipid-derived volatiles as determined by DHE and most notably 2,4-decadienal, a characteristic odour impact compound in chicken (Chen et al., 2019). Although the change in aroma profile surprisingly did not enhance the oily note determined by the trained panel during

quantitative descriptive analysis (QDA), the chicken and roasted character was significantly augmented, which was suggested to have masked the chicken broth aroma. This was because the introduction of phospholipids did not give rise to a significant increase in the concentration of S-containing Maillard reaction products with cooked vegetable and potato odours, such as methional, methanethiol and the methyl sulfides, which potentially contributed to the chicken broth character described. Meanwhile, an aqueous dispersion of egg yolk phospholipids heated at 100 °C for 20 min was described to have strong oily and chicken broth attributes, as compared to the generic meaty aroma found in that containing freeze-dried egg yolk with trace lipids, or the eggy and egg yolk broth notes found in the natural egg yolk dispersion (Yang et al., 2022). The results from the instrumental analysis correlated with that from the sensory evaluation as there was a significantly higher concentration of lipid-derived volatiles in the sample with egg yolk phospholipids, although the lack of inhibition of the Maillard reaction was also reported. Confounding factors were considered in this study and it was shown that lutein, which is a predominant xanthophyll pigment in egg yolk, acted as a fat-soluble antioxidant by scavenging free radicals in the system and thereby, retarding lipid oxidation as well as the Maillard reaction. This aptly exemplifies of how natural constituents of starting materials could affect the reactivity of phospholipids and therefore aroma generation.

Despite the limited number of studies examining the effect of phospholipids on meat aroma generation, as well as the differences in experimental conditions and matrices ranging from model systems to meat products, the clear consensus is that the addition of phospholipids to a system enhances the production of lipid-derived volatiles. While this is hardly surprising, the impact on the aroma quality of the samples has to be determined with consideration of the odour thresholds of these compounds and thus,

sensory evaluation can be a useful tool to complement instrumental analysis. On the other hand, observations of the effect of phospholipids on the Maillard reaction were more disparate. This could be in part due to the different ratios of the reactants present in the system. In the aqueous model reactions, the phospholipids were present at 1.5 – 2 times higher than the combined quantities of sugars and amino acids, while in the meat matrices, the available quantities were close to natural concentrations and unlikely to be higher than these other precursors. Moreover, the presence of endogenous meat constituents such as pro- and anti-oxidants even at trace levels, as well as native proteins, could also have profound effects on the complex aroma formation mechanisms. In addition, the heating conditions of the model systems were usually higher and carried out under pressure while milder boiling temperatures were employed when meat was involved. Furthermore, the use of HS-SPME-GC-MS analysis in the latest study could potentially be hindered by matrix effects due to the different retention capacities of volatiles in samples with different formulations, as compared to the more exhaustive DHE.

1.5 Conclusion

The literature on the oxidative properties of different phospholipids shows a wide variation of results which has made the comparison of the reactivity of phospholipids a challenging task. The divergent conclusions drawn about the prooxidative and antioxidative properties of phospholipids among these studies were inevitable due to the variation in experimental designs inducing different activities in the phospholipids and other system components. Thus, it is of great pertinence to assess the often heterogenous molecular environment of the phospholipids when performing studies which involve this highly sensitive and dynamic class of compounds. In the context of a meat matrix, the concentration of endogenous prooxidants and antioxidants, as well

as the presence and structural state of native myofibrillar and sarcoplasmic proteins, could influence the rate and extent of phospholipid oxidation and ultimately aroma generation.

As a consequence of the changing behaviour of phospholipids in distinct environments, there is no straightforward conclusion to their impact on meat aroma generation, except for an inevitable increase in the concentration of lipid-derived volatiles upon their addition to a system while the concomitant effect on Maillard or lipid-Maillard reaction products was more obscure. The diverse classes of aroma compounds, which possess unique properties and formation pathways, present in meat matrices only adds another layer of complexity to the investigation. During meat aroma generation, it could be possible that phospholipids exert both pro- and anti-oxidative effects via parallel reaction mechanisms and the net impact would be dependent on individual matrix and reaction conditions.

The use of model systems of sugars and amino acids has proven to be a useful starting point for our incipient understanding of the role of phospholipids in meat aroma generation. To take into account the complex heterogeneity of meat systems, future investigations could explore the progressive addition of meat constituents, including but not limited to transition metal ions (e.g., Fe^{2+}), peptides or proteins (e.g., myosin, myoglobin), vitamins, minerals and enzymes, to model systems at a pH relevant to meat (usually pH 5.5 – 6). This would facilitate the deconvolution of the effects of interacting parameters and determination of the impact of phospholipids on meat aroma generation with less ambiguity. Alternatively, freeze-dried meat matrices could offer a convenient and natural source of precursors for the reactions to take place, although a characterisation of the composition of the system would be ideal.

Furthermore, the molecular organisation of the system could be studied to understand its impact on precursor degradation and volatile formation.

While this review has highlighted the importance of phospholipids in meat aroma generation, there is no doubt that the elucidation of the role of phospholipids is still an area worthy of further research. There are many outstanding aspects of phospholipid oxidation and its interaction with the Maillard reaction remaining to be unravelled but with adequate consideration of the influence of the confounding factors discussed. This knowledge will be useful for food manufacturers who are keen on lipid innovation in new product development, especially within the plant-based meat category, including but not limited to the creation and modification of process meat flavourings in products for applications in soups, bouillons and meat analogues.

1.6 References

Akutsu, H. (2020). Structure and dynamics of phospholipids in membranes elucidated by combined use of NMR and vibrational spectroscopies. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1862*(9), 183352.

Amaral, A. B., da Silva, M. V., & Lannes, S. C. d. S. (2018). Lipid oxidation in meat: Mechanisms and protective factors - A review. *Food Science and Technology, 38*, 1-15.

Amrani-Hemaimi, M., Cerny, C., & Fay, L. B. (1995). Mechanisms of formation of alkylpyrazines in the maillard reaction. *Journal of Agricultural and Food Chemistry, 43*(11), 2818-2822.

Applegate, K. R., & Glomset, J. A. (1986). Computer-based modeling of the conformation and packing properties of docosahexaenoic acid. *J Lipid Res, 27*(6), 658-680.

Araseki, M., Yamamoto, K., & Miyashita, K. (2002). Oxidative stability of polyunsaturated fatty acid in phosphatidylcholine liposomes. *Bioscience, Biotechnology, and Biochemistry, 66*(12), 2573-2577.

Ballance, P. E. (1961). Production of volatile compounds related to the flavour of foods from the Strecker degradation of DL-methionine. *Journal of the Science of Food and Agriculture, 12*(7), 532-536.

Berton-Carabin, C. C., Ropers, M.-H., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. *Comprehensive reviews in food science and food safety, 13*(5), 945-977.

Blank, I., Davidek, T., Devaud, S., Sagalowicz, L., Leser, M. E., & Michel, M. (2006). Formation of 4-hydroxy-5-methyl-3(2H)-furanone (norfuraneol) in structured fluids. In W. L. P. Bredie & M. A. Petersen (Eds.), *Developments in Food Science* (Vol. 43, pp. 347-350): Elsevier.

Brands, C. M. J., & van Boekel, M. A. J. S. (2001). Reactions of monosaccharides during heating of sugar−casein systems: Building of a reaction network model. *Journal of Agricultural and Food Chemistry, 49*(10), 4667-4675.

Bueschelberger, H.-G., Tirok, S., Stoffels, I., & Schoeppe, A. (2015). Lecithins. In V. Norn (Ed.), *Emulsifiers in Food Technology* (2nd ed., pp. 21-60). West Sussex: John Wiley & Sons.

Cardenia, V., Waraho, T., Rodriguez-Estrada, M. T., McClements, J. D., & Decker, E. A. (2011). Antioxidant and prooxidant activity behavior of phospholipids in stripped soybean oil-in-water emulsions. *Journal of the American Oil Chemists' Society, 88*(9), 1409-1416.

Cerny, C. (2008). The aroma side of the Maillard reaction. *Annals of the New York Academy of Sciences, 1126*(1), 66-71.

Cerny, C., & Davidek, T. (2003). Formation of aroma compounds from ribose and cysteine during the maillard reaction. *Journal of Agricultural and Food Chemistry, 51*(9), 2714-2721.

Chen, D.-W., Balagiannis, D. P., & Parker, J. K. (2019). Use of egg yolk phospholipids to generate chicken meat odorants. *Food Chemistry, 286*, 71-77.

Corliss, G. A., & Dugan Jr., L. R. (1970). Phospholipid oxidation in emulsions. *Lipids, 5*(10), 846-853.

Cui, L., & Decker, E. A. (2016). Phospholipids in foods: prooxidants or antioxidants? *Journal of the Science of Food and Agriculture, 96*(1), 18-31.

Cullis, P. R., & De Kruijff, B. (1979). Lipid polymorphism and the functional roles of lipids in biological membranes. *Biochimica et Biophysica Acta (BBA) - Reviews on Biomembranes, 559*(4), 399-420.

Dacaranhe, C. D., & Terao, J. (2001). Effect of phosphatidic acid and phosphatidylserine on lipid oxidation in beef homogenate during storage and in emulsified sardine oil. *Journal of Food Science, 66*(3), 422-427.

Doert, M., Jaworska, K., Moersel, J. T., & Kroh, L. W. (2012). Synergistic effect of lecithins for tocopherols: Lecithin-based regeneration of α-tocopherol. *European Food Research and Technology, 235*(5), 915-928.

Doig, S. D., & Diks, R. M. M. (2003). Toolbox for modification of the lecithin headgroup. *European Journal of Lipid Science and Technology, 105*(7), 368-376.

Dominguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants (Basel), 8*(10), 429.

Dowhan, W. (1997). Molecular basis for membrane phospholipid diversity: Why are there so many lipids? *Annual Review of Biochemistry, 66*(1), 199-232.

Erickson, M. C. (2008). Chemistry and function of phospholipids. In C. C. Akoh & D. B. Min (Eds.), *Food Lipids: Chemistry, Nutrition and Biotechnology* (3rd ed., pp. 39-62). Boca Raton: CRC Press.

Eshuis, J. J. W. (2011). World Patent No. WO2011039155 A1. International Bureau: World Intellectual Property Organization.

Fahy, E., Subramaniam, S., Brown, H. A., et al. (2005). A comprehensive classification system for lipids. *J Lipid Res, 46*(5), 839-862.

Farmer, L. J., & Mottram, D. S. (1990). Interaction of lipid in the maillard reaction between cysteine and ribose: The effect of a triglyceride and three phospholipids on the volatile products. *Journal of the Science of Food and Agriculture, 53*(4), 505-525.

Farmer, L. J., Mottram, D. S., & Whitfield, F. B. (1989). Volatile compounds produced in maillard reactions involving cysteine, ribose and phospholipid. *Journal of the Science of Food and Agriculture, 49*(3), 347-368.

Fujimaki, M., Kato, S., & Kurata, T. (1969). Pyrolysis of sulfur-containing amino acids. *Agricultural and Biological Chemistry, 33*(8), 1144-1151.

Gasser, U., & Grosch, W. (1990). Primary odorants of chicken broth. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 190*(1), 3-8.

Gray, J. I. (1978). Measurement of lipid oxidation: A review. *Journal of the American Oil Chemists' Society, 55*(6), 539-546.

Hanahan, D. J., Turner, M. B., & Jayko, M. E. (1951). The isolation of egg phosphatidyl choline by an adsorption column technique. *Journal of Biological Chemistry, 192*(2), 623-628.

Hardman, P. D. (1982). Spin-label characterisation of the lamellar-to-hexagonal (HII) phase transition in egg phosphatidylethanolamine aqueous dispersions. *European Journal of Biochemistry, 124*(1), 95-101.

Harlos, K., & Eibl, H. (1980). Influence of calcium on phosphatidylethanolamine. An investigation of the structure at high pH. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 601*, 113-122.

Hatanaka Hiroji, H. (1991). US Patent No. US 5028449 A. U.S. Patent and Trademark Office.

He, J., Evans, N. M., Liu, H., & Shao, S. (2020). A review of research on plant-based meat alternatives: Driving forces, history, manufacturing, and consumer attitudes. *Comprehensive reviews in food science and food safety, 19*(5), 2639-2656.

Hidalgo, F. J., Nogales, F., & Zamora, R. (2005). Changes produced in the antioxidative activity of phospholipids as a consequence of their oxidation. *Journal of Agricultural and Food Chemistry, 53*(3), 659-662.

Hidalgo, F. J., & Zamora, R. (2004). Strecker-type degradation produced by the lipid oxidation products 4,5-epoxy-2-alkenals. *Journal of Agricultural and Food Chemistry, 52*(23), 7126-7131.

Hodge, J. E. (1953). Chemistry of browning reactions in model systems. *Journal of Agricultural and Food Chemistry, 1*(15), 928-943.

Huang, Y.-C., Li, H.-J., He, Z.-F., Wang, T., & Qin, G. (2010). Study on the flavor contribution of phospholipids and triglycerides to pork. *Food Science and Biotechnology, 19*(5), 1267-1276.

Igene, J. O., & Pearson, A. M. (1979). Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *Journal of Food Science, 44*(5), 1285-1290.

Jansen, F. J. H. M. (2014). Managing flavour changes during storage. In J. K. Parker, S. Elmore, & L. Methven (Eds.), *Flavour Development, Analysis and Perception in Food and Beverages* (pp. 249-270). Cambridge: Woodhead Publishing.

Jayasena, D. D., Ahn, D. U., Nam, K. C., & Jo, C. (2013). Flavour chemistry of chicken meat: A review. *Asian-Australasian Journal of Animal Sciences, 26*(5), 732-742.

Joshi, A., Paratkar, S. G., & Thorat, B. N. (2006). Modification of lecithin by physical, chemical and enzymatic methods. *European Journal of Lipid Science and Technology, 108*(4), 363-373.

Kato, T., Hirukawa, T., & Namiki, K. (1992). Selective terminal olefin oxidation of n-3 polyunsaturated fatty acids. *Tetrahedron letters, 33*(11), 1475-1478.

Kawakatsu, M., Terao, J., & Matsushita, S. (1984). Phospholipid oxidation catalyzed by ferrous ion and ascorbic acid. *Agricultural and Biological Chemistry, 48*(5), 1275-1279.

Kerth, C. R., & Miller, R. K. (2015). Beef flavor: A review from chemistry to consumer. *Journal of the Science of Food and Agriculture, 95*(14), 2783-2798.

Ladikos, D., & Lougovois, V. (1990). Lipid oxidation in muscle foods: A review. *Food Chemistry, 35*(4), 295-314.

Lawrie, R. A., & Ledward, D. A. (2006). Chemical and biochemical constitution of muscle. In R. A. Lawrie & D. A. Ledward (Eds.), *Lawrie's Meat Science* (7th ed., pp. 75-127). Cambrdge: Woodhead Publishing.

Lee, E. C., & Tandy, J. S. (1991). USA Patent No. US5039543. Washington, DC: U.S. Patent and Trademark Office.

Lehninger, A. L., Nelson, D. L., & Cox, M. M. (2012). Lipids. In *Principles of Biochemistry* (6th ed., pp. 343-368). New York: Macmillan Learning.

Liang, L., Chen, F., Wang, X., Jin, Q., Decker, E. A., & McClements, D. J. (2017). Physical and oxidative stability of flaxseed oil-in-water emulsions fabricated from sunflower lecithins: Impact of blending lecithins with different phospholipid profiles. *Journal of Agricultural and Food Chemistry, 65*(23), 4755-4765.

Lin, J., & Blank, I. (2003). Odorants generated by thermally induced degradation of phospholipids. *Journal of Agricultural and Food Chemistry, 51*(15), 4364-4369.

Lin, J., Leser, M. E., Löliger, J., & Blank, I. (2004). A new insight into the formation of odor active carbonyls by thermally-induced degradation of phospholipids in self-assembly structures. *Journal of Agricultural and Food Chemistry, 52*(3), 581-586.

Livingston, D. J., & Brown, W. D. (1981). The chemistry of myoglobin and its reactions. *Food Technology*(35), 244.

Lordan, R., Tsoupras, A., & Zabetakis, I. (2017). Phospholipids of animal and marine origin: Structure, function, and anti-inflammatory properties. *Molecules, 22*(11), 1964.

Lu, F. S. H., Nielsen, N. S., Baron, C. P., Diehl, B. W. K., & Jacobsen, C. (2013). Impact of primary amine group from aminophospholipids and amino acids on marine phospholipids stability: Non-enzymatic browning and lipid oxidation. *Food Chemistry, 141*(2), 879-888.

Lu, F. S. H., Nielsen, N. S., Baron, C. P., & Jacobsen, C. (2012). Oxidative degradation and non-enzymatic browning due to the interaction between oxidised lipids and primary amine groups in different marine PL emulsions. *Food Chemistry, 135*(4), 2887-2896.

Lu, F. S. H., Nielsen, N. S., Baron, C. P., & Jacobsen, C. (2017). Marine phospholipids: The current understanding of their oxidation mechanisms and potential uses for food fortification. *Critical Reviews in Food Science and Nutrition, 57*(10), 2057-2070.

Luzzati, V., & Tardieu, A. (1974). Lipid phases: Structure and structural transitions. *Annual Review of Physical Chemistry, 25*(1), 79-94.

Macy, R. L., Naumann, H. D., & Bailey, M. E. (1964). Water-soluble flavor and odor precursors of meat. I. Qualitative study of certain amino acids, carbohydrates, non-amino acid nitrogen compounds, and phosphoric acid esters of beef, pork, and lamb. *Journal of Food Science, 29*(2), 136-141.

Mancuso, J. R., McClements, D. J., & Decker, E. A. (1999). The effects of surfactant type, pH, and chelators on the oxidation of salmon oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 47*(10), 4112-4116.

Mandigo, R. W., & Sullivan, G. A. (2014). Chemistry and physics of comminuted products - Emulsions and Batters. In M. Dikeman & C. Devine (Eds.), *Encyclopedia of Meat Sciences* (2nd ed., pp. 283-288). Oxford: Academic Press.

Mantsch, H. H., & McElhaney, R. N. (1991). Phospholipid phase transitions in model and biological membranes as studied by infrared spectroscopy. *Chemistry and Physics of Lipids, 57*(2), 213-226.

Martins, S. I. F. S., Jongen, W. M. F., & van Boekel, M. A. J. S. (2000). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology, 11*(9), 364-373.

Mcclements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science, 65*(8), 1270-1282.

Mei, L., McClements, D. J., Wu, J., & Decker, E. A. (1998). Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl. *Food Chemistry, 61*(3), 307-312.

Min, B., & Ahn, D. U. (2005). Mechanism of lipid peroxidation in meat and meat products - A review. *Food Science and Biotechnology, 14*(1), 152-163.

Miyashita, K., Azuma, G., & Ota, T. (1995). Oxidative stability of geometric and positional isomers of unsaturated fatty acids in aqueous solution. *Journal of Japan Oil Chemists' Society, 44*(6), 425-430.

Miyashita, K., Nara, E., & Ota, T. (1993). Oxidative stability of polyunsaturated fatty acids in an aqueous solution. *Bioscience, Biotechnology, and Biochemistry, 57*(10), 1638-1640.

Miyashita, K., Nara, E., & Ota, T. (1994). Comparative study on the oxidative stability of phosphatidylcholines from salmon egg and soybean in an aqueous solution. *Bioscience, Biotechnology, and Biochemistry, 58*(10), 1772-1775.

Moon, J. K., & Shibamoto, T. (2009). Antioxidant assays for plant and food components. *Journal of Agricultural and Food Chemistry, 57*(5), 1655-1666.

Mottram, D. S. (1995). Maillard-lipid interactions in nonaqueous systems: Volatiles from the reaction of cysteine and ribose with phosphatidylcholine. *Journal of Agricultural and Food Chemistry, 43*(5), 1302-1306.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415-424.

Mottram, D. S. (2007). The Maillard Reaction: Source of Flavour in Thermally Processed Foods. In R. G. Berger (Ed.), *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability* (pp. 269-283). Berlin, Heidelberg: Springer Berlin Heidelberg.

Mottram, D. S., & Edwards, R. A. (1983). The role of triglycerides and phospholipids in the aroma of cooked beef. *Journal of the Science of Food and Agriculture, 34*(5), 517-522.

Mozuraityte, R., Kristinova, V., Rustad, T., & Storrø, I. (2016). The role of iron in peroxidation of PUFA: Effect of pH and chelators. *European Journal of Lipid Science and Technology, 118*(4), 658-668.

Nara, E., Miyashita, K., & Ota, T. (1997). Oxidative stability of liposomes prepared from soybean PC, chicken egg PC, and salmon egg PC. *Bioscience, Biotechnology, and Biochemistry, 61*(10), 1736-1738.

Parker, J. K. (2014). Thermal generation of aroma. In J. K. Parker, S. Elmore, & L. Methven (Eds.), *Flavour Development, Analysis and Perception in Food and Beverages* (pp. 151-185). Cambridge: Woodhead Publishing.

Parker, J. K. (2017). Meat. In A. Buettner (Ed.), *Springer Handbook of Odor* (pp. 191-222). Cham, Switzerland: Springer.

Pichot, R., Watson, R. L., & Norton, I. T. (2013). Phospholipids at the interface: Current trends and challenges. *International Journal of Molecular Sciences, 14*(6), 11767-11794.

Pripis-Nicolau, L., de Revel, G., Bertrand, A., & Maujean, A. (2000). Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. *Journal of Agricultural and Food Chemistry, 48*(9), 3761-3766.

Raes, K., & De Smet, S. (2009). Fatty Acids. In L. M. L. Nollet & F. Toldra (Eds.), *Handbook of Muscle Foods Analysis* (pp. 141-154). Boca Raton: CRC Press.

Reis, A., & Spickett, C. M. (2012). Chemistry of phospholipid oxidation. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1818*(10), 2374-2387.

Rizzi, G. P. (2008). The strecker degradation of amino acids: Newer avenues for flavor formation. *Food Reviews International, 24*(4), 416-435.

Ruiz, J., Muriel, E., Perez-Palacios, T., & Antequera, T. (2009). Analysis of phospholipids in muscle foods. In L. M. L. Nollet & F. Toldra (Eds.), *Handbook of Muscle Foods Analysis* (pp. 167-186). Boca Raton: CRC Press.

Salter, L. J., Mottram, D. S., & Whitfield, F. B. (1988). Volatile compounds produced in maiilard reactions involving glycine, ribose and phospholipid. *Journal of the Science of Food and Agriculture, 46*(2), 227-242.

Samdani, G. K., McClements, D. J., & Decker, E. A. (2018). Impact of phospholipids and tocopherols on the oxidative stability of soybean oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 66*(15), 3939-3948.

Schneider, M. (1989). Fractionation and purification of lecithin. In B. F. Szuhaj (Ed.), *Lecithins: Sources, Manufacture & Uses* (1st ed., pp. 109-130). Champaign, Illinois: American Oil Chemists' Society.

Seddon, J. M., & Cevc, G. (1993). Lipid polymorphism: Structure and stability of lyotropic mesophases of phospholipids. In G. Cevc (Ed.), *Phospholipids Handbook* (1st ed., pp. 403-454). New York: Marcel Dekker, Inc.

Seddon, J. M., Cevc, G., Kaye, R. D., & Marsh, D. (1984). X-ray diffraction study of the polymorphism of hydrated diacyl and dialkylphosphatidylethanolamines. *Biochemistry, 23*(12), 2634-2644.

Shibamoto, T., & Bernhard, R. A. (1977). Investigation of pyrazine formation pathways in glucose-ammonia model systems. *Agricultural and Biological Chemistry, 41*(1), 143-153.

Szuhaj, B. F. (1983). Lecithin production and utilization. *Journal of the American Oil Chemists' Society, 60*(2Part1), 306-309.

Tafesse, F. G., Ternes, P., & Holthuis, J. C. M. (2006). The multigenic sphingomyelin synthase family. *Journal of Biological Chemistry, 281*(40), 29421-29425.

Tatulian, S. A. (1993). Ionization and ion binding. In G. Cevc (Ed.), *Phospholipids Handbook* (1st ed., pp. 511-552). New York: Marcel Dekker, Inc.

Timmons, M., Ngo, H., Ryan, D. A., & Dariush, A. (2021). WO2021142157A1. International Bureau: World Intellectual Property Organization.

Tims, M. J., & Watts, B. M. (1958). Relationship of meat pigments to fat oxidation. *Food Technology, 1958*(12), 240-243.

Tressl, R., Nittka, C., Kersten, E., & Rewicki, D. (1995). Formation of isoleucine-specific maillard products from [1-13C]-D-glucose and [1-13C]-D-fructose. *Journal of Agricultural and Food Chemistry, 43*(5), 1163-1169.

van Boekel, M. A. J. S. (2001). Kinetic aspects of the Maillard reaction: a critical review. *Food / Nahrung, 45*(3), 150-159.

van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances, 24*(2), 230-233.

van Nieuwenhuyzen, W. (1976). Lecithin production and properties. *Journal of the American Oil Chemists' Society, 53*(6Part2), 425-427.

van Nieuwenhuyzen, W. (1981). The industrial uses of special lecithins: A review. *Journal of the American Oil Chemists Society, 58*(10), 886-888.

Vauthey, S., Milo, C., Frossard, P., Garti, N., Leser, M. E., & Watzke, H. J. (2000). Structured fluids as microreactors for flavor formation by the maillard reaction. *Journal of Agricultural and Food Chemistry, 48*(10), 4808-4816.

Wang, G., & Wang, T. (2008). Oxidative stability of egg and soy lecithin as affected by transition metal ions and pH in emulsion. *Journal of Agricultural and Food Chemistry, 56*(23), 11424-11431.

Waraho, T., Cardenia, V., Rodríguez-Estrada, M. T., McClements, D. J., & Decker, E. A. (2009). Prooxidant mechanisms of free fatty acids in stripped soybean oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 57*(15), 7112-7117.

Waraho, T., McClements, D. J., & Decker, E. A. (2011). Impact of free fatty acid concentration and structure on lipid oxidation in oil-in-water emulsions. *Food Chemistry, 129*(3), 854-859.

Weihrauch, J. L., & Son, Y. S. (1983). Phospholipid content of foods. *Journal of the American Oil Chemists' Society, 60*(12), 1971-1978.

Whitfield, F. B., Mottram, D. S., Brock, S., Puckey, D. J., & Salter, L. J. (1988). Effect of phospholipid on the formation of volatile heterocyclic compounds in heated aqueous solutions of amino acids and ribose. *Journal of the Science of Food and Agriculture, 42*(3), 261-272.

Willemot, C., Poste, L. M., Salvador, J., Wood, D. F., & Butler, G. (1985). Lipid degradation in pork during warmed-over flavour development. *Canadian Institute of Food Science and Technology Journal, 18*(4), 316-322.

Wu, Y., Chen, Z., Jia, J., Chiba, H., & Hui, S. P. (2021). Quantitative and comparative investigation of plasmalogen species in daily foodstuffs. *Foods, 10*(1), 124.

Yang, X., Liu, J., Wan, P., Guo, D., & Chen, D. W. (2022). Use of egg yolk to imitate meat aroma. *Food Chemistry, 371*, 131112.

Yin, M. C., & Faustman, C. (1993). Influence of temperature, pH, and phospholipid composition upon the stability of myoglobin and phospholipid: A liposome model. *Journal of Agricultural and Food Chemistry, 41*(6), 853-857.

Yu, T. H., & Ho, C. T. (1995). Volatile compounds generated from thermal reaction of methionine and methionine sulfoxide with or without glucose. *Journal of Agricultural and Food Chemistry, 43*(6), 1641-1646.

Zhang, Y., & Ho, C. T. (1991). Comparison of the volatile compounds formed from the thermal reaction of glucose with cysteine and glutathione. *Journal of Agricultural and Food Chemistry, 39*(4), 760-763.

1.7 Tables and figures

1.7.1 Tables

Table 1-1: Average composition of plant and animal lecithins (Bueschelberger et al., 2015)

Phospholipid	% of total phospholipids					
	Soy	**Rapeseed**	**Sunflower**	**Corn**	**Egg yolk**	**Milk**
PC	24	25	25	30	74	27
PE	22	22	11	3	19	36
PS	15	15	19	16	1	-
PI	7	-	4	9	-	-
SM	-	-	-	-	2	29

PC= Phosphatidylcholine; PE= Phosphatidylethanolamine; PS= Phosphatidylserine; PI= Phosphatidylinositol; SM= Sphingomyelin

1.7.2 Figures

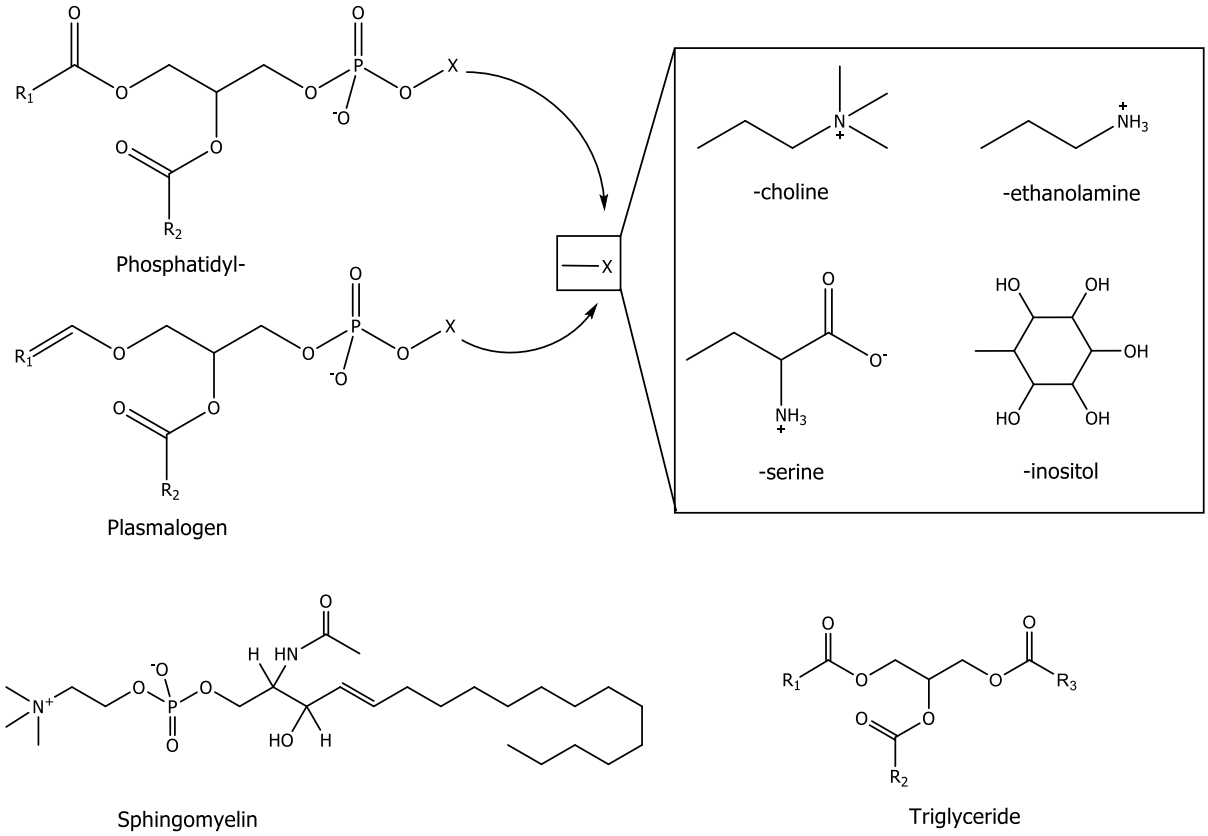


Figure 1-1: Chemical structure of phospholipids and triglyceride

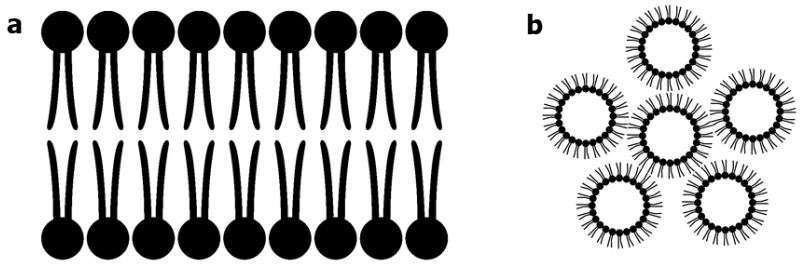


Figure 1-2: Physical structures of phospholipids (a: lamellar, L_α; b: inverse hexagonal, H_{II}) (Adapted from Erickson (2008) and Cullis and De Kruijff (1979))

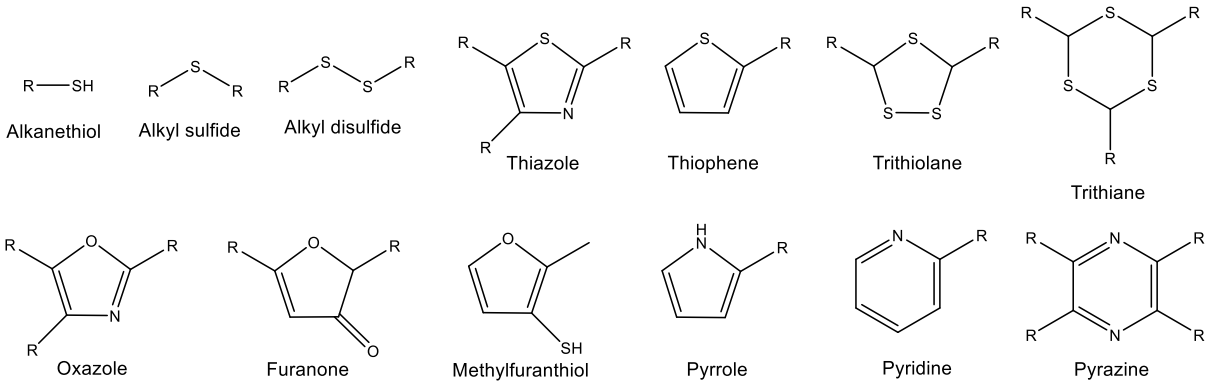


Figure 1-3: Classes of volatile compounds formed during Maillard reaction (Reprinted with permission from Mottram (1998). Copyright 1998 Elsevier)

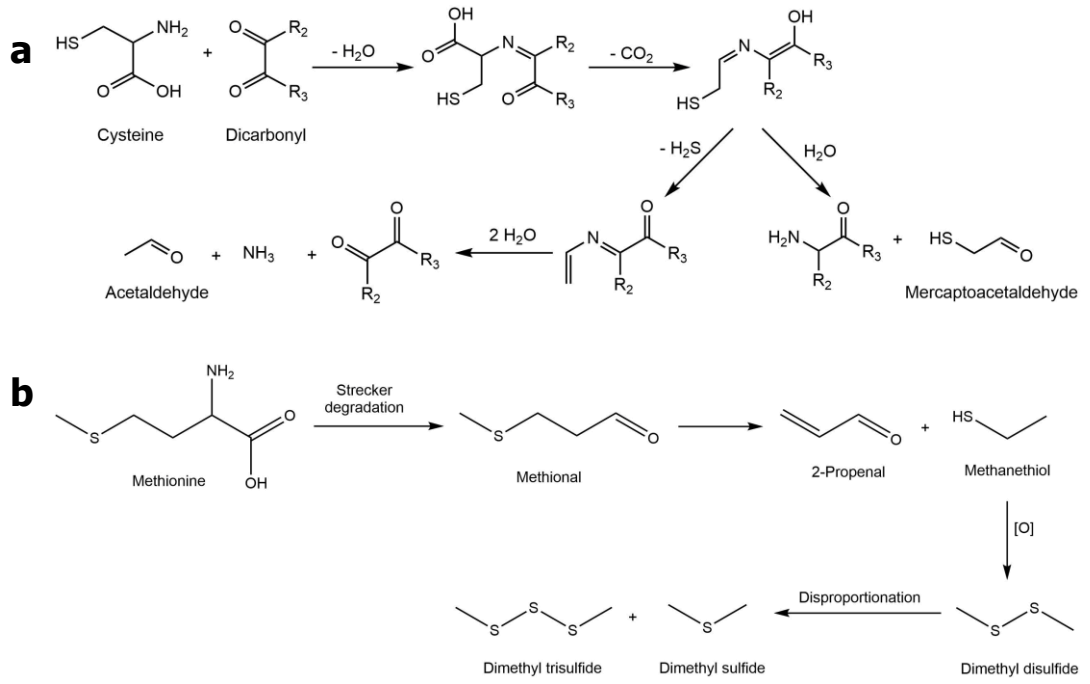


Figure 1-4: Strecker degradation of a) cysteine and b) methionione (Reprinted with permission from Parker (2017). Copyright 2017 Springer Nature)

2. Comparison of odorants in beef and chicken broth

Abstract

Aroma extracts of boiled chicken and beef were isolated using Likens-Nickerson simultaneous distillation-extraction (SDE). The in-situ heating of the sample facilitates the capture of aroma intermediates during the cooking process, thereby mimicking the cooking of meat in stocks and stews. The extracts were then analysed by Gas Chromatography-Mass Spectrometry (GC-MS) and GC-Olfactometry (GC-O). Most of the volatiles identified using GC-O were sulfur-containing compounds, such as sulfides, thiols and mercaptoaldehydes, which are derived from the Maillard reaction. Meanwhile, lipid oxidation resulted in the formation of unsaturated aldehydes, such as alkenals and alkadienals. Families of thiazoles and thiazolines were also found in the extracts. Novel thiazolines, 5-ethyl-2,4-dimethyl-3-thiazoline and 2-ethyl-4,5-dimethyl-3-thiazoline, were identified in this work and synthesised in the laboratory from their respective aldehyde and mercaptoketone precursors for the confirmation of identity. The differences in ratios of key aroma volatiles in boiled beef and chicken could contribute towards their characteristic odours.

Keywords: Beef, chicken, GC-O, thiazole, thiazoline

2.1 Introduction

The rapid growth in vegan, vegetarian and flexitarian diets has led to renewed interest in the development of process flavourings with authentic flavour profiles in search of potential vegan solutions. Despite the advances in analytical techniques, recent studies were more interested in specific animal breeds (Delort et al., 2011; Fan et al., 2018; Frank et al., 2016; Inagaki et al., 2017), distinct animal parts (Delort et al., 2011; Straßer & Schieberle, 2014) or special cooking techniques (Christlbauer & Schieberle, 2009; Schindler et al., 2010) rather than a comparison between species using the same cooking and extraction technique.

The characteristic aroma of cooked meat is generated from thermally induced reactions, principally the Maillard reaction and lipid oxidation, between non-volatile components of lean and adipose tissues during heating. These complex reaction pathways lead to the formation of a diverse range of products, accounting for the large number of volatile compounds found in cooked meat. While the aroma compounds produced during the Maillard reaction are typically responsible for the savoury, meaty (e.g. S-containing compounds such as 2-methyl-3-furanthiol), roast (e.g. pyrazines such as 2-ethyl-3,5/6-dimethylpyrazine) and boiled characters, those derived from lipid oxidation (e.g. saturated and unsaturated aldehydes such as 2-alkenals and 2,4-alkadienals) impart fatty aromas to cooked meat and can contribute to species-specific notes (Mottram, 1998).

The aim of this study is to isolate volatiles of boiled chicken (BC) and boiled beef (BB) using Likens-Nickerson simultaneous distillation-extraction (LN–SDE). Despite artifact formation being a drawback of LN–SDE due to the thermal input, this technique offers the advantage of in-situ heating and extraction, which facilitates the mimicking of meat cooking in stocks and stews in a realistic kitchen setting and avoids the loss of aroma during sample transfer

inevitable in other methods. Equipped with more powerful analytical techniques as compared to decades ago, our knowledge of the key volatiles present in these meat aromas could be updated and an understanding of the differences between these species based on a fair comparison (i.e. same cooking and extraction technique) will be useful in the creation and modification of process flavourings in products such as soups, stocks and bouillons.

2.2 Materials and methods

2.2.1 Reagents and chemicals

Aroma chemicals were obtained from the following suppliers and were ≥95% in purity unless stated otherwise: acetaldehyde, hexanal, heptanal, octanal, nonanal, decanal, 3-(methylthio)propanal, 2-methylbutanal, (E)-2-nonenal, (E,E)-2,4-heptadienal (90%), (E,E)-2,4,-nonadienal (≥89%), (E,E)-2,6-nonadienal, 1-octen-3-ol, (Z)-4-heptenal, 1-octen-3-one, 2,3-butanedione, 2,3-pentanedione, 3-mercapto-2-butanone, dimethyl disulfide, dimethyl trisulfide, benzothiazole, 2-methyl-3-furyl methyl disulfide, 2-acetylpyrrole, 2-acetyl-2-thiazoline, tetramethylpyrazine and 2-isopropylpyrazine were from Sigma Aldrich (Gillingham, UK); 4-nonanone, 2-furylmethanethiol, 3-mercapto-2-pentanone, 2-methyl-2-thiazoline, 1-(methylthio)propane, 2-ethyl-4,5-dimethyl-3-thiazoline from TCI (Oxford, UK); (E,E)-2,4-decadienal (90%) and trimethyloxazole from Lancaster Synthesis (Heysham, UK); phenylacetaldehyde and 3-octen-2-one from Acros Organics (New Jersey, USA); 1-(methylthio)ethanethiol from Carbosynth (Newbury, UK); 1-propanethiol from Fisher Scientific (Loughborough, UK); furfuryl methyl disulfide, 2,4-dimethylthiazole from Oxford Organics; 2-acetylthiazole from Riverside Aromatics (Poole, UK); 2-ethyl-3,5-dimethylpyrazine from Fluorochem (Hadfield, UK); 3-methylbutanal from Alfa Aeser (Heysham, UK); 2-methyl-3-furanthiol from IFF (Haverhill, UK) and nonanoic acid from Anitox (Wellingborough, UK). Pentane (≥98%), diethyl ether (≥99.5%), dichloromethane (≥99.8%), ammonium sulfide (20% wt. solution in H_2O), propanal (97%), and 1-hydroxy-2-butanone (Aldrich^{CPR};

purity unknown) were from Sigma-Aldrich. 1-Hydroxy-2-propanone (97%) was from Fluka (Seelze, Germany). High purity water (18.2 MΩ) was obtained from a Select Fusion Ultrapure water deionisation unit (SUEZ, Peterborough, UK).

2.2.2 Likens-Nickerson simultaneous distillation-extraction (LN-SDE)

Fresh beef silverside joint and fresh Class A chicken breast were purchased from a retail supermarket and used within the sell-by date. The meats were provided by one commercial supplier and from the same batch and farm. The chickens were a standard Ross 308 genotype and were reared, slaughtered and processed under commercial conditions. The meat was trimmed of extramuscular fat and minced using a food mincer with 4.5 mm screen (Kenwood, Hampshire, UK). The minced meat (500g) and high purity water (500g) were added to a round bottom flask. The sample was boiled at 100 °C in a heating mantle for 30 min. Likens-Nickerson SDE was performed using 30 mL of redistilled pentane/diethyl ether (9:1 v/v) for 2 h. The extract was concentrated to 0.5 mL using a Vigreux column and stored at -80 °C prior to analysis.

2.2.3 Gas-Chromatography Olfactometry (GC-O)

GC-O analyses were performed on a HP 5890 Series II GC equipped with a flame ionisation detector (Hewlett Packard, Waldbronn, BaWü, Germany) and an ODO II odour port (SGE, Ringwood, Victoria, Australia). An aliquot of sample (2 µL) was injected in splitless mode. Chromatographic separation was achieved on two columns of different polarities. For the non-polar Rxi-5 Sil MS column (30 m x 0.25 mm x 1 µm; Restek, Bellefonte, PA, USA), the oven temperature was programmed from 35 °C to 200 °C at 6 °C min^{-1} and finally to 320 °C at 15 °C min^{-1}. For the polar ZB-Wax column (30 m x 0.25 mm x 0.25 µm; Phenomenex, Torrance, CA, USA), the oven temperature was programmed from 35 °C to 200 °C at 6 °C min^{-1} and finally to 250 °C at 15 °C min^{-1}. All initial and final temperatures were held for 10 min. Helium

was used as the carrier gas at a constant flow rate of 2.0 mL min^{-1}. The column effluent was split equally between the FID and odour port, where the odours of the eluting components were evaluated. The samples were analysed in duplicates by 3 trained assessors who were screened for olfactory performance in terms of threshold, discrimination and identification using a Sniffin' Sticks test (Burghardt®, Wedel, Germany). Each has a global olfactory score of ≥ 38 and at least one year of GC-O experience. The descriptions and intensity scores (on a scale of 1 – 10, where 3 = weak, 5 = medium and 7 = strong) for the odours detected were recorded.

2.2.4 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analyses were performed on an Agilent 7890A GC coupled to an Agilent 5975C inert XL EI/CI MSD triple axis MS (Agilent Technologies, Santa Clara, CA, USA). An aliquot of sample (2 µL) was injected in splitless mode. Chromatographic separation was achieved on two columns of different polarities. For the non-polar HP-5 MS column (30 m x 0.25 mm x 1 µm; Agilent Technologies), the oven temperature was increased from 35 °C to 320 °C at 6 °C min^{-1}. For the polar ZB-Wax column (60 m x 0.25 mm x 0.25 µm; Phenomenex), the oven temperature was increased from 35 °C to 250 °C at 4 °C min^{-1}. All initial and final temperatures were held for 10 min. The carrier gas was helium at a constant pressure of 18 psi. The MS was operated in electron impact mode with a source temperature of 250 °C, ionisation energy of 70 eV and a scan range from m/z 29 to m/z 400.

2.2.5 Compound identification

The chromatograms were processed using Agilent MSD ChemStation (version F.01.03.2365; Agilent Technologies). A series of C_5 – C_{25} *n*-alkanes was analysed under the same conditions for the calculation of the linear retention index (LRI) of each compound. The identities of the compounds were confirmed based on a match of their mass spectra, LRI and odour descriptors

with those of standard compounds where available. Otherwise, a tentative identification was made by comparing their mass spectrum against the NIST 14 library, as well as LRI and odour descriptors available in literature.

2.2.6 3-Thiazoline and thiazole synthesis

Synthesis was carried out using an adapted method from Elmore and Mottram (1997). Equimolar quantities (5 mmol) of an aldehyde and a mercaptoketone or α-hydroxyketone depending on availability (Table 2-1) were added to 50 mL 0.1 M ammonium sulfide solution in a Duran bottle before heating in a 100 °C water bath for 30 min with constant stirring using a magnetic stirrer. The reaction mixtures were allowed to cool at room temperature before extraction with 30 mL dichloromethane. The extracts were flushed under nitrogen before storage at -20 °C until analysis by GC-MS as described in Section 2.2.4. As the purpose was to obtain a mass spectrum and LRI for identification, these extracts were not purified.

2.3 Results and discussion

2.3.1 Odorants in BB and BC

The aroma extracts of both BB and BC had an intense aroma characteristic of their respective species and the volatiles identified by GC-O and GC-MS for BB and BC extracts are listed in Table 2-2 and Table 2-3 respectively. A total of 58 and 65 odour-active volatiles were identified in BB and BC respectively. The major classes of compounds in both extracts comprised S- and/or N-containing compounds, including heterocycles such as thiazoles, thiazolines and pyrazines, as well as straight chain saturated and unsaturated aldehydes, alcohols and ketones.

2.3.1.1 S-containing compounds

Many of the early-eluting odorants (LRI < 500 on DB-5 column) were highly potent S-containing compounds with very high volatilities, namely hydrogen sulfide, methanethiol,

ethanethiol and 2-propanethiol. Thus, some were not detected by GC-MS and only by GC-O as the human nose can be a more sensitive detector. Nevertheless, the majority of the compounds with the highest odour intensity scores and detection frequencies in both the extracts were S-containing compounds, such as 2-methyl-3-furanthiol (cooked meat, roasted), 2-furylmethanethiol (roasted, burnt) and 3-(methylthio)propanal (potato), which were among the primary odorants identified in chicken broth (Gasser & Grosch, 1990). As observed for these compounds, not all S-containing compounds impart meaty odours. In fact, it was reported that furans and thiophenes with a thiol group in the 3-position, and related disulfides, such as compounds containing 2-methyl-3-furanyl moieties, possess strong meat-like notes, while those with 2-methylfuranyl moieties had roast, nutty, onion and burnt characteristics (Evers et al., 1976; Farmer & Patterson, 1991; Mottram et al., 1995).

Sulfur compounds play a major role in meat aroma as they usually have low odour threshold values and their diversity is manifested in the various formation pathways possible between sulfur precursors and reducing sugars in the Maillard reaction. One of the sources of sulfur comes from the free amino acid methionine, which generates 3-(methylthio)propanal as a Strecker degradation product (Ballance, 1961; Fujimaki et al., 1969). Besides contributing to aroma profile, 3-(methylthio)propanal also serves as a precursor for other S-containing volatiles due to its instability, and subsequently breaks down to form methanethiol (Yu & Ho, 1995), which readily oxidises to dimethyl disulfide (Pripis-Nicolau et al., 2000). Another important precursor is cysteine, which yields acetaldehyde, hydrogen sulfide and ammonia as reactive species upon Strecker degradation (Fujimaki et al., 1969). The interaction of hydrogen sulfide with dicarbonyls, furanones and furfurals produces thiols and mercaptoketones, which can form a mixture of disulfides upon oxidation (Mottram, 1998). More specifically, the reaction between hydrogen sulfide and 4-hydroxy-5-methyl-3(2H)-furanone (norfuraneol) or furan-2-aldehyde, which are the major degradation products of pentoses, produces 2-methyl-3-furanthiol (Hofmann & Schieberle, 1998; Whitfield & Mottram,

1999) or 2-furylmethanethiol (Hofmann & Schieberle, 1998; Münch et al., 1997) respectively. Alternative pathways for the formation of 2-methyl-3-furanthiol via 1,4-deoxypentosulose (Cerny & Davidek, 2003) and thiamin degradation (Guentert et al., 1990) have also been proposed. In the presence of both S-containing amino acids, 1-(methylthio)ethanethiol can also be formed from acetaldehyde, methanethiol and hydrogen sulfide, which are the Strecker degradation products of cysteine and methionine (Schutte & Koenders, 1972).

2.3.1.2 N-containing compounds

The reactive intermediates generated during the Maillard reaction also participate in pyrazine formation. The condensation of two α-aminocarbonyl compounds, derived from the Strecker degradation, gives rise to dihydropyrazines as intermediates, which form pyrazines upon oxidation (Shibamoto & Bernhard, 1977). Tetramethylpyrazine could be produced from the reaction between 2,3-butanedione and amino acids during the Strecker degradation, generating 3-amino-2-butanone as an intermediate, which dimerises to tetramethyldihydropyrazine and forms the pyrazine upon oxidation (Huang et al., 1996). An alternative and more reactive mechanism involves the reaction between 2,3-butanedione, methylglyoxal and formaldehyde (Low et al., 2007). For the more substituted pyrazines, the methyl and ethyl substituent groups can be derived from formaldehyde and acetaldehyde respectively, and these short-chain aldehydes are the degradation products of glycine and alanine respectively (Amrani-Hemaimi et al., 1995). In the case of 2-ethyl-3,5-dimethylpyrazine, the Strecker degradation involving alanine and pyruvaldehyde (sugar degradation product) yields 2-aminopropanal and aminoacetone, which form 2,6-dimethyldihydropyrazine on condensation and the reaction of this intermediate with acetaldehyde yields the pyrazine (Cerny & Grosch, 1994). An alternative pathway for pyrazine formation via deamination has also been proposed (Shu, 1998).

In addition to amino-containing compounds, the liberation of ammonia during the Strecker degradation also introduces a nitrogen source to the system for the formation of N-containing heterocycles, such as thiazolines and thiazoles, discussed in the next section.

2.3.1.3 Other Maillard reaction compounds

Besides the generation of a pool of reactive precursors, some of the aldehydes formed in the Strecker degradation also contribute to the aroma of both the extracts. 3-Methylbutanal (malty, cocoa, nutty), 2-methylbutanal (chocolate liquor, aldehydic) and phenylacetaldehyde (floral, rose, perfume, green) are derived from leucine, isoleucine (Pripis-Nicolau et al., 2000) and phenylalanine (Hwang et al., 1995) respectively.

2.3.1.4 Lipid oxidation products

Carbonyl compounds derived from the oxidative degradation of unsaturated fatty acids were also identified in both extracts. (E,E)-2,4-decadienal (fatty, fried), (E)-2-nonenal (fatty, aldehydic, waxy), 1-octen-3-one (cooked mushroom) and (E,E)-2,4-nonadienal (fatty) could be formed from the autoxidation of n-6 fatty acids such as linoleate (Cossignani et al., 2014) and arachidonate (Blank, 2000; Taylor & Mottram, 1990). Meanwhile, aldehydes such as octanal (fruity, sweet) and nonanal (fatty, citral, leafy) could originate from the autoxidation of n-9 fatty acids such as oleate. Although none of these carbonyl compounds identified possessed aroma characteristics resembling the complete spectrum of cooked chicken (Pippen & Nonaka, 1960), it was reported that the removal of carbonyl compounds from the volatile fraction resulted in a loss of chicken odour and an intensification of meaty odour (Minor et al., 1965). However, it was also demonstrated that the omission of (E,E)-2,4-decadienal alone did not result in a significant aroma change (Feng et al., 2018). Thus, it is likely that it is the delicate balance of the group of carbonyl compounds which contribute to the overall aroma rather than a single aroma compound alone.

2.3.2 Comparison between BB and BC extracts

Comparing between the two species, there were 21 volatiles identified in the BB extract which were not found in the BC extract and 14 volatiles vice versa. Although the majority of the volatiles were present in both the extracts, the odour intensities of these compounds varied, indicating their different levels of contribution to the aroma of each extract.

More notably, bis(2-methyl-3-furyl)disulfide (beef fat, meaty, fatty) was only found in the BB extract. This compound, with an extremely low odour threshold value of 2.4×10^{-5} μg kg^{-1} in water (Buttery et al., 1984), is a dimer of 2-methyl-3-furanthiol (Ruther & Baltes, 1994) and was also found in several other studies (Abd El-Aleem et al., 2017; Christlbauer & Schieberle, 2009; Kerscher & Grosch, 1997). In addition, 3-(methylthio)propanal and 2-phenylacetaldehyde were ranked at higher total intensities of 45.5 and 25.5 respectively in the BB extract as compared to 33 and 20 respectively in the BC extract. These results corroborated with the findings of Gasser and Grosch (1990) that bis(2-methyl-3-furyl)disulfide and the Strecker aldehydes were more predominant in the aroma of boiled beef. Furthermore, these authors also reported 12-methyltridecanal to be a beef-specific odorant with tallowy, beef-like notes, which was proposed to be liberated from plasmalogens in ruminants after a long heating period (Guth & Grosch, 1993, 1994). However, this branched aldehyde was not found in our study, which could be attributed to the milder heating conditions and shorter duration impeding the release of the aldehyde from plasmalogens. From a survey of literature, it was apparent that 12-methyltridecanal was usually detected in studies where the heating conditions involved long hours at high temperatures (e.g. 200 °C for 4 h) (Christlbauer & Schieberle, 2009; Guth & Grosch, 1993, 1994) and not in those with shorter times (Cerny & Grosch, 1992; Gasser & Grosch, 1990; Kerler & Grosch, 1996; Schindler et al., 2010; Specht & Baltes, 1994). An interesting exception was the presence of 12-methyltridecanal in Japanese

Wagyu beef and Australian grass-fed beef although the meat was only heated at 80 °C for 2 min (Inagaki et al., 2017). This could be due to the thinly sliced form of the meat providing an increased surface area for heat transfer or the higher levels of the branched aldehyde naturally present in the plasmalogens of these breeds as it has been shown that pasture feeding significantly increased the aldehyde concentrations up to 350 times in the muscle phospholipids of bulls (Dannenberger et al., 2006). Another explanation would be that the trace occurrence of 12-methyltridecanal in the aroma extracts was too low for detection (Grosch, 1994).

Some fatty odorants, such as (E,E)-2,4-heptadienal and (E,E)-2,4-nonadienal, were only found in the BC extract, while others such as (E)-2-heptenal (green, citral, waxy), (Z)-2-nonenal (fatty, plastic-like) and (E,E)-2,6-nonadienal (violets, floral, waxy, fatty) were only found in the BB extract. However, the strength of fatty odours in the overall aroma is not a consequence of the quantity of identified aldehydes and the odour activities of the volatiles ought to be considered (Table 2-4). Thus, it was possible that fatty odorants had a greater contribution to BC than BB aroma, as reported by Gasser and Grosch (1990) who observed a stronger prevalence of fatty odorants such as (E,E)-2,4-decadienal, γ-dodecalactone and (E/Z)-2-undecenal in the aroma of BC as compared to BB as a result of the higher unsaturated fat content in the former. Although (E,E)-2,4-decadienal was present in both extracts, the odour intensity was almost twice as strong in the BC extract as compared to the BB extract.

2.3.3 3-Thiazolines and thiazoles

3-Thiazolines were first reported in cooked beef and the odour descriptions of nutty, roasted, meaty, onion and vegetable-like, depending on the substituents, were similar to the closely-related thiazoles although compounds from both classes with the same alkyl substituents might not necessarily have the same odours (Mussinan et al., 1976). Their

existence in fried chicken (Tang et al., 1983), wheat flour (Bredie et al., 2002) and sesame seed oil (Agyemang et al., 2011a) have also been documented and patents have also been filed for the use of 3-thiazolines in flavour applications (Agyemang et al., 2011b; Spencer et al., 1982). However, less than 10 have been approved for use in flavouring substances (European Food Safety Authority). On the other hand, thiazoles were more commonly found and have been mentioned in chicken and beef broths (Guth & Grosch, 1994), fried chicken (Tang et al., 1983), roast beef (Chaintreau et al., 2006), as well as model systems containing cysteine, ribose and phospholipid (Farmer et al., 1989). The formation pathway was proposed to be from the reaction of α-hydroxyketones or α-dicarbonyls with hydrogen sulfide and ammonia in the presence of aliphatic aldehydes, yielding 3-thiazolines which form thiazoles upon oxidation (Elmore & Mottram, 1997). In addition to the α-hydroxyketones and α-dicarbonyls, the presence of mercaptoketones, which are intermediates in the mechanism, could also result in the formation of thiazolines and thiazoles. Since these precursors were identified in the GC-MS and GC-O, the formation of thiazolines and thiazoles in the BB and BC extracts was possible.

Several 3-thiazolines and thiazoles were identified in this study, among which 2,4-dimethyl-3-thiazoline (meaty, brothy, roasted), 2,4-dimethylthiazole (meaty, grilled chicken, roasted), trimethyl-3-thiazoline (pickled onion, cat's pee; meaty, fried onion), and 5-ethyl-2,4-dimethylthiazole (popcorn, nutty, sweet and roasted) had been found in beef with odour thresholds of 0.02, 0.5, 0.1 and 0.002 mg kg^{-1} in water respectively (Mussinan et al., 1976). The last two compounds were also found in fried chicken, along with 4-ethyl-2-methylthiazole (popcorn, basmati, biscuit, roasting skin) (Tang et al., 1983). These authors also suggested that the lack of mention of 3-thiazolines in literature is due to their presence in trace quantities and susceptibility to oxidation. The presence of thiazolines also exists in other foods such as 2-ethyl-4,5-dimethyl-3-thiazoline (roasted onion, rice) in freeze-dried onion sprout (Takahashi

& Shibamoto, 2008), trimethylisothiazole (odd sulfur, cardboard, earthy, green) in yeast extract paste (Mahadevan & Farmer, 2006) and sesame seed oil (Agyemang et al., 2011a). A new 3-thiazoline, 5-ethyl-2,4-dimethyl-3-thiazoline (fatty, grilled meat, savoury), was reported in BB and BC extracts for the first time. The mass spectra of the pair of stereoisomers are provided in Table 2-5 while their formation mechanisms and mass fragmentation patterns are proposed in Figure 2-1 and Figure 2-2 respectively. Given the high odour intensity scores of these compounds, they could also be important contributors to boiled meat aromas.

Among all the 3-thiazolines and thiazoles with confirmed identities (i.e. by odour description, GC-O and GC-MS LRIs), 2,4-dimethyl-3-thiazoline, 4-ethyl-2-methylthiazole and 5-ethyl-2,4-dimethylthiazole were only identified in BC. These compounds share a common roast aroma, with the 3-thiazoline possessing a meaty note and the thiazoles bearing sweeter or more fragrant attributes such as popcorn, nutty, basmati and barley. The toasted and rice qualities could also be enhanced in BC by the higher odour intensity score of 2-acetylthiazole in BC than BB (24 v.s. 19.5). Meanwhile, the 3-thiazolines present in BB were characterised by stronger savoury (onion and meaty) notes as manifested in the higher odour intensity scores for 5-ethyl-2,4-dimethyl-3-thiazoline (33 in BB v.s. 20 in BC) and trimethyl-3-thiazoline isomer I (30.5 in BB v.s. 17 in BC). Overall, 3-thiazolines and thiazoles contributed to the toast, nutty and rice notes in the BC aroma to create a more rounded profile, while the onion and meaty attributes could be enhanced in the BB aroma to produce a more intense savoury perception.

2.4 Conclusion

Although the majority of the volatiles were present in both extracts, each still possessed its characteristic odour, indicating that it was the delicately balanced contribution of each volatile, as exemplified in the different odour intensities, which brings the complex and nuanced aroma profile of BB and BC into harmony. Nevertheless, the trends were that S-containing

compounds and Strecker aldehydes were more prevalent in BB than BC, while fatty odorants, which were mainly the lipid-derived aldehydes, were more predominant in BC than BB. In addition, this study has demonstrated the important contribution of 3-thiazolines in boiled meat aroma, which may have been overlooked in the past.

2.5 References

Abd El-Aleem, F. S., Taher, M. S., Lotfy, S. N., El-Massry, K. F., & Fadel, H. H. M. (2017). Influence of extracted 5-nucleotides on aroma compounds and flavour acceptability of real beef soup. *International Journal of Food Properties, 20*(sup1), S1182-S1194.

Agyemang, D., Bardsley, K., Brown, S., Kraut, K., Psota-Kelty, L., & Trinnaman, L. (2011a). Identification of 2-ethyl-4-methyl-3-thiazoline and 2-isopropyl-4-methyl-3-thiazoline for the first time in nature by the comprehensive analysis of sesame seed oil. *J Food Sci, 76*(3), C385-391.

Agyemang, D. O., Bardsley, K. A., Psota-Kelty, L., & Trinnama, L. (2011b). USA Patent No. US20110052775 Al. Washington, DC: U.S. Patent and Trademark Office.

Amrani-Hemaimi, M., Cerny, C., & Fay, L. B. (1995). Mechanisms of formation of alkylpyrazines in the maillard reaction. *Journal of Agricultural and Food Chemistry, 43*(11), 2818-2822.

Ballance, P. E. (1961). Production of volatile compounds related to the flavour of foods from the Strecker degradation of DL-methionine. *Journal of the Science of Food and Agriculture, 12*(7), 532-536.

Blank, I., Lin, J., & Fay, L.B. (2000). Aroma impact compounds formed by autoxidation of arachidonic acid. In P. Schieberle, and Engel, K. H. (Ed.), *Frontiers of Flavour Science* (pp. 3-9). Garching: Deutsche Forschungsanstalt für Lebensmittelchemie.

Bredie, W. L. P., Mottram, D. S., & Guy, R. C. E. (2002). Effect of temperature and pH on the generation of flavor volatiles in extrusion cooking of wheat flour. *Journal of Agricultural and Food Chemistry, 50*(5), 1118-1125.

Buttery, R. G., Haddon, W. F., Seifert, R. M., & Turnbaugh, J. G. (1984). Thiamin odor and bis(2-methyl-3-furyl) disulfide. *Journal of Agricultural and Food Chemistry, 32*(3), 674-676.

Buttery, R. G., Turnbaugh, J. G., & Ling, L. C. (1988). Contribution of volatiles to rice aroma. *Journal of Agricultural and Food Chemistry, 36*(5), 1006-1009.

Cerny, C., & Davidek, T. (2003). Formation of aroma compounds from ribose and cysteine during the maillard reaction. *Journal of Agricultural and Food Chemistry, 51*(9), 2714-2721.

Cerny, C., & Grosch, W. (1992). Evaluation of potent odorants in roasted beef by aroma extract dilution analysis. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 194*(4), 322-325.

Cerny, C., & Grosch, W. (1994). Precursors of ethyldimethylpyrazine isomers and 2,3-diethyl-5-methylpyrazine formed in roasted beef. *Z Lebensm Unters Forsch, 198*(3), 210-214.

Chaintreau, A., Rochat, S., & de Saint Laumer, J. Y. (2006). Re-investigation of sulfur impact odorants in roast beef using comprehensive two-dimensional GC-TOF-MS and the GC-SNIF technique. In W. L. P. Bredie & M. A. Petersen (Eds.), *Developments in Food Science* (Vol. 43, pp. 601-604): Elsevier.

Christlbauer, M., & Schieberle, P. (2009). Characterization of the key aroma compounds in beef and pork vegetable gravies á la chef by application of the aroma extract dilution analysis. *Journal of Agricultural and Food Chemistry, 57*(19), 9114-9122.

Cossignani, L., Giua, L., Simonetti, M. S., & Blasi, F. (2014). Volatile compounds as indicators of conjugated and unconjugated linoleic acid thermal oxidation. *European Journal of Lipid Science and Technology, 116*(4), 407-412.

Dannenberger, D., Lorenz, S., Nuernberg, G., Scollan, N., Ender, K., & Nuernberg, K. (2006). Analysis of fatty aldehyde composition, including 12-methyltridecanal, in plasmalogens from longissimus muscle of concentrate- and pasture-fed bulls. *Journal of Agricultural and Food Chemistry, 54*(1), 182-188.

Delort, E., Velluz, A., Frérot, E., et al. (2011). Identification and synthesis of new volatile molecules found in extracts obtained from distinct parts of cooked chicken. *Journal of Agricultural and Food Chemistry, 59*(21), 11752-11763.

Elmore, J. S., & Mottram, D. S. (1997). Investigation of the reaction between ammonium sulfide, aldehydes, and α-hydroxyketones or α-dicarbonyls to form some lipid-Maillard interaction products found in cooked beef. *Journal of Agricultural and Food Chemistry, 45*(9), 3595-3602.

European Food Safety Authority. Food Flavourings Database (Publication no. https://webgate.ec.europa.eu/foods_system/main/?sector=FFL&auth=SANCAS). Retrieved 24th April 2022

Evers, W. J., Heinsohn, H. H., Mayers, B. J., & Sanderson, A. (1976). Furans substituted at the three position with sulfur. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors* (Vol. 26, pp. 184-193): American Chemical Society.

Fan, M., Xiao, Q., Xie, J., et al. (2018). Aroma compounds in chicken broths of Beijing Youji and commercial broilers. *Journal of Agricultural and Food Chemistry, 66*(39), 10242-10251.

Farmer, L. J., Mottram, D. S., & Whitfield, F. B. (1989). Volatile compounds produced in maillard reactions involving cysteine, ribose and phospholipid. *Journal of the Science of Food and Agriculture, 49*(3), 347-368.

Farmer, L. J., & Patterson, R. L. S. (1991). Compounds contributing to meat flavour. *Food Chemistry, 40*(2), 201-205.

Feng, Y., Cai, Y., Fu, X., Zheng, L., Xiao, Z., & Zhao, M. (2018). Comparison of aroma-active compounds in broiler broth and native chicken broth by aroma extract dilution analysis (AEDA), odor activity value (OAV) and omission experiment. *Food Chemistry, 265*, 274-280.

Flaig, M., Qi, S., Wei, G., Yang, X., & Schieberle, P. (2020). Characterization of the key odorants in a high-grade chinese green tea beverage (Camellia sinensis; Jingshan cha) by means of the sensomics approach and elucidation of odorant changes in tea leaves caused by the tea manufacturing process. *Journal of Agricultural and Food Chemistry, 68*(18), 5168-5179.

Frank, D., Ball, A., Hughes, J., et al. (2016). Sensory and flavor chemistry characteristics of Australian beef: Influence of intramuscular fat, feed, and breed. *Journal of Agricultural and Food Chemistry, 64*(21), 4299-4311.

Fujimaki, M., Kato, S., & Kurata, T. (1969). Pyrolysis of sulfur-containing amino acids. *Agricultural and Biological Chemistry, 33*(8), 1144-1151.

Gasser, U., & Grosch, W. (1990). Primary odorants of chicken broth. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 190*(1), 3-8.

Grosch, W. (1994). Determination of potent odourants in foods by Aroma Extract Dilution Analysis (AEDA) and calculation of Odour Activity Values (OAVs). *Flavour and Fragrance Journal, 9*(4), 147-158.

Guentert, M., Bruening, J., Emberger, R., et al. (1990). Identification and formation of some selected sulfur-containing flavor compounds in various meat model systems. *Journal of Agricultural and Food Chemistry, 38*(11), 2027-2041.

Guth, H., & Grosch, W. (1993). 12-Methyltridecanal, a Species-Specific Odorant of Stewed Beef. *LWT - Food Science and Technology, 26*(2), 171-177.

Guth, H., & Grosch, W. (1994). Identification of the character impact odorants of stewed beef juice by instrumental analyses and sensory studies. *Journal of Agricultural and Food Chemistry, 42*(12), 2862-2866.

Hofmann, T., & Schieberle, P. (1998). Quantitative model studies on the effectiveness of different precursor systems in the formation of the intense food odorants 2-furfurylthiol and 2-methyl-3-furanthiol. *Journal of Agricultural and Food Chemistry, 46*(1), 235-241.

Huang, T. C., Fu, H. Y., & Ho, C. T. (1996). Mechanistic studies of tetramethylpyrazine formation under weak acidic conditions and high hydrostatic pressure. *Journal of Agricultural and Food Chemistry, 44*(1), 240-246.

Hwang, H. I., Hartman, T. G., & Ho, C. T. (1995). Relative reactivities of amino acids in pyrazine formation. *Journal of Agricultural and Food Chemistry, 43*(1), 179-184.

Inagaki, S., Amano, Y., & Kumazawa, K. (2017). Identification and characterization of volatile components causing the characteristic flavor of Wagyu beef (Japanese black cattle). *Journal of Agricultural and Food Chemistry, 65*(39), 8691-8695.

Kerler, J., & Grosch, W. (1996). Odorants contributing to warmed-over flavor (wof) of refrigerated cooked beef. *Journal of Food Science, 61*(6), 1271-1275.

Kerscher, R., & Grosch, W. (1997). Comparative evaluation of potent odorants of boiled beef by aroma extract dilution and concentration analysis. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A, 204*(1), 3-6.

Low, M. Y., Parker, J. K., & Mottram, D. S. (2007). Mechanisms of alkylpyrazine formation in a potato model system containing added glycine. *Journal of Agricultural and Food Chemistry, 55*(10), 4087-4094.

Mahadevan, K., & Farmer, L. (2006). Key odor impact compounds in three yeast extract pastes. *Journal of Agricultural and Food Chemistry, 54*(19), 7242-7250.

Milo, C., & Grosch, W. (1993). Changes in the odorants of boiled trout (Salmo fario) as affected by the storage of the raw material. *Journal of Agricultural and Food Chemistry, 41*(11), 2076-2081.

Minor, L. J., Pearson, A. M., Dawson, L. E., & Schweigert, B. S. (1965). Separation and identification of carbonyl and sulfur compounds in volatile fraction of cooked chicken. *Journal of Agricultural and Food Chemistry, 13*(4), 298-300.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415-424.

Mottram, D. S., Madruga, M. S., & Whitfield, F. B. (1995). Some novel meatlike aroma compounds from the reactions of alkanediones with hydrogen sulfide and furanthiols. *Journal of Agricultural and Food Chemistry, 43*(1), 189-193.

Münch, P., Hofmann, T., & Schieberle, P. (1997). Comparison of key odorants generated by thermal treatment of commercial and self-prepared yeast extracts: Influence of the amino acid composition on odorant formation. *Journal of Agricultural and Food Chemistry, 45*(4), 1338-1344.

Mussinan, C. J., Wilson, R. A., Katz, I. R. A., Hruza, A., & Vock, M. H. (1976). Identification and flavor properties of some 3-oxazolines and 3-thiazolines isolated from cooked beef. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors* (Vol. 26, pp. 133-145): American Chemical Society.

Pippen, E. L., & Nonaka, M. (1960). Volatile carbonyl compounds of cooked chicken. II. Compounds volatilized with steam during cooking. *Journal of Food Science, 25*(6), 764-769.

Pripis-Nicolau, L., de Revel, G., Bertrand, A., & Maujean, A. (2000). Formation of flavor components by the reaction of amino acid and carbonyl compounds in mild conditions. *Journal of Agricultural and Food Chemistry, 48*(9), 3761-3766.

Ruther, J., & Baltes, W. (1994). Sulfur-containing furans in commercial meat flavorings. *Journal of Agricultural and Food Chemistry, 42*(10), 2254-2259.

Schindler, S., Krings, U., Berger, R. G., & Orlien, V. (2010). Aroma development in high pressure treated beef and chicken meat compared to raw and heat treated. *Meat Science, 86*(2), 317-323.

Schutte, L., & Koenders, E. B. (1972). Components contributing to beef flavor. Natural precursors of 1-methylthioethanethiol. *Journal of Agricultural and Food Chemistry, 20*(2), 181-184.

Shibamoto, T., & Bernhard, R. A. (1977). Investigation of pyrazine formation pathways in glucose-ammonia model systems. *Agricultural and Biological Chemistry, 41*(1), 143-153.

Shu, C. K. (1998). Pyrazine formation from amino acids and reducing sugars, a pathway other than strecker degradation. *Journal of Agricultural and Food Chemistry, 46*(4), 1515-1517.

Specht, K., & Baltes, W. (1994). Identification of volatile flavor compounds with high aroma values from shallow-fried beef. *Journal of Agricultural and Food Chemistry, 42*(10), 2246-2253.

Spencer, M. D., Parliment, T. H., & Giordano, D. A. (1982). U.S Patent No. 4355049. Washington, DC: U.S. Patent and Trademark Office.

Straßer, S., & Schieberle, P. (2014). Characterization of the key aroma compounds in roasted duck liver by means of aroma extract dilution analysis: comparison with beef and pork livers. *European Food Research and Technology, 238*(2), 307-313.

Takahashi, M., & Shibamoto, T. (2008). Chemical compositions and antioxidant/anti-inflammatory activities of steam distillate from freeze-dried onion (Allium cepa L.) sprout. *Journal of Agricultural and Food Chemistry, 56*(22), 10462-10467.

Tang, J., Jin, Q. Z., Shen, G. H., Ho, C. T., & Chang, S. S. (1983). Isolation and identification of volatile compounds from fried chicken. *Journal of Agricultural and Food Chemistry, 31*(6), 1287-1292.

Taylor, A. J., & Mottram, D. S. (1990). Composition and odour of volatiles from autoxidised methyl arachidonate. *Journal of the Science of Food and Agriculture, 50*(3), 407-417.

Whitfield, F. B., & Mottram, D. S. (1999). Investigation of the reaction between 4-hydroxy-5-methyl-3(2h)-furanone and cysteine or hydrogen sulfide at pH 4.5. *Journal of Agricultural and Food Chemistry, 47*(4), 1626-1634.

Yu, T. H., & Ho, C. T. (1995). Volatile compounds generated from thermal reaction of methionine and methionine sulfoxide with or without glucose. *Journal of Agricultural and Food Chemistry, 43*(6), 1641-1646.

2.6 Tables and figures

2.6.1 Tables

Table 2-1: Precursors for 3-thiazoline and thiazole synthesis

Reactants		**Products**
Aldehyde	α-Hydroxyketone/ Mercaptoketone	3-Thiazoline and thiazole
Acetaldehyde	1-Hydroxy-2-propanone	2,4-Dimethyl-
Acetaldehyde	1-Hydroxy-2-butanone	4-Ethyl-2-methyl-
Acetaldehyde	3-Mercapto-2-pentanone	5-Ethyl-2,4-dimethyl-
Propanal	3-Mercapto-2-butanone	2-Ethyl-4,5-dimethyl-
Acetaldehyde	3-Mercapto-2-butanone	Trimethyl-

Table 2-2: Odour active compounds in the boiled beef (BB) extract

Compound	Odour description[a]	Intensity[b]	Detection frequency[c]	LRI_{DB-5}[d]		LRI_{ZB-Wax}[e]		ID[f]
				GC-O	GC-MS	GC-O	GC-MS	
Hydrogen sulfide	Poo, rotten egg, sulfur	31	6	<500	<500	<800	n.d.	O, ms
Acetaldehyde	Sweet solvent	9	3	<500	<500	<800	n.d.	O, ms, lri
Methanethiol	Roast, sulfur, rotten cabbage, poo	34.5	6	<500	<500	<800	n.d.	O, ms, lri
Ethanethiol	Town gas, sulfurous	18	4	<500	n.d.	<800	n.d.	O, lri
2-Propanethiol	Raw onion	26.5	4	<500	n.d.	n.d.	n.d.	O, lri
Formic acid	Mustard, horseradish, brassica	36	5	<500	n.d.	n.d.	n.d.	O, lri
2,3-Butanedione	Buttery, cheesy, sweaty	35	6	574	n.d.	987	987	O, MS, LRI
1-Propanethiol	Raw onion, musty, meaty	13	4	603	608	n.d.	n.d.	O, MS, LRI
3-Methylbutanal	Malty, cocoa, nutty	35.5	6	646	644	921	919	O, MS, LRI
2-Methylbutanal	Chocolate liquor, aldehydic	7	3	655	654	914	915	O, MS, LRI
2,3-Pentanedione	Creamy, buttery, cheesy, sweaty	30.5	6	696	696	1061	1068	O, MS, LRI
Methanedithiol	Raw/ rotting onion, catty, petroleum	38	6	737	n.d.	n.d.	n.d.	O, lri
Dimethyl disulfide	Sulfurous, vegetable, cough syrup	22.5	6	745	744	1080	1072	O, MS, LRI
1-(Methylthio)propane	Onion, pungent, paint, petrol	36	6	766	763	n.d.	n.d.	O, MS
Hexanal	Green, fatty, grassy	36	6	799	801	1097	1082	O, MS, LRI
Mercaptopropanone	Vegetable, sulfur, garlic, beer headspace	28	6	805	n.d	1352	n.d.	O, MS, LRI
Unknown	Brothy, meaty	10	2	813	-	-	-	-
3-Mercapto-2-butanone	Vegetable, sulfur, pyrazine, cardboard	8	2	817	818	1270	n.d.	O, MS, LRI
3-Methyl-2-butene-1-thiol	Stewed onion, garlic, beer headspace, meaty	25.5	6	823	n.d	n.d.	n.d.	O, lri
1-(Methylthio)ethanethiol + Ethyl methyl disulfide	Rotting onions, sulfur, catty, petroleum	38	6	845	847	1221	n.d.	O, MS, LRI
				845	848	1142	1134	O, ms, lri
2-Methyl-3-furanthiol	Roasted, porridge oats, sulfur, faecal	41	6	866	872	1303	n.d.	O, MS, LRI
Trimethyloxazole	Popcorn, nutty, vegetable	20	5	869	851	1150	n.d.	O, MS, LRI
2-Methyl-2-thiazoline	Boiled/rotting onion, catty, sulfur, medicinal	22	5	876	873	1275	n.d.	O, MS, LRI
2,4-Dimethylthiazole	Meaty, brassica	11	3	890	889	1270	1283	O, MS, LRI

Compound	Odour description[a]	Intensity[b]	Detection frequency[c]	LRI_{DB-5}[d]		LRI_{ZB-Wax}[e]		ID[f]
				GC-O	GC-MS	GC-O	GC-MS	
(Z)-4-Heptenal	Lamb fat, potato, meaty, buttery	48.5	6	895	900	1234	n.d.	O, MS, LRI
3-Mercapto-2-pentanone	Onion, catty	20	4	898	903	1346	n.d.	O, MS, LRI
Methional	Potato	45.5	6	901	909	1451	1467	O, MS, LRI
2-Furylmethanethiol + 2-Mercapto-3-pentanone	Stewed potato, meaty, beef, gravy	39	5	905 905	912 n.d.	1430 -	1432 -	O, MS, LRI O, lri
2-Acetyl-1-pyrroline	Basmati rice	42	6	918	924	1322	1327	O, MS, LRI
Propyl 2-methylbutanoate	Perfume, fruity, saffron	11	3	947	944	n.d.	n.d.	O, ms, lri
(E)-2-heptenal	Green, citral, waxy	17	4	961	961	1318	1311	O, MS, LRI
Unknown	Fruity, cat's pee, blackcurrant	8.5	2	963	-	-	-	-
Dimethyl trisulfide	Meaty, onion	11	2	967	976	1362	1360	O, MS, LRI
1-Octen-3-one + 1-Octen-3-ol	Raw mushroom, green, flower	39	6	976 976	982 982	1291 1449	1284 1451	O, MS, LRI O, MS, LRI
(Z)-1,5-Octadien-3-one	Geranium	21	4	978	n.d	n.d	n.d.	O, lri
2-Methyl-3-octanone	Vegetable, earth, plastic, garlic	19	3	987	986	n.d.	n.d.	O, ms, lri
Trimethyl-3-thiazoline (I)	Pickled onion, cat's pee	30.5	5	990	990	1367	1363	O, MS, LRI
Trimethyl-3-thiazoline (II)	Meaty, fried onion	16	3	994	992	1373	1380	O, MS, LRI
Octanal	Sweet, fruity, star burst candy	24.5	4	999	1005	1279	1265	O, MS, LRI
Trimethylisothiazole	Odd sulfur, cardboard, earthy, green	32	6	1010	1008	1395	n.d.	ms, lri
2-Acetylthiazole	Toasted, biscuit, basmati rice	19.5	5	1021	1024	1648	1657	O, MS, LRI
3-Octen-2-one	Earthy, plastic	11	3	1039	1041	1376	n.d.	O, MS, LRI
Phenylacetaldehyde	Floral, rose, green	25.5	6	1047	1052	1644	1656	O, MS, LRI
2-Acetyl-1,4,5,6-tetrahydropyridine	Popcorn, fried rice	13	3	1056	1053	-	-	O, ms, lri
2-Ethyl-3,5-dimethylpyrazine	Cardboard, pyrazine, cocoa, soil	24	5	1075	1076	n.d.	n.d.	O, MS, LRI
2-Ethyl-4,5-dimethyl-3-thiazoline (I)	Roasted onion/garlic	5	1	1083	1094	n.d.	n.d.	MS, LRI
5-Ethyl-2,4-dimethyl-3-thiazoline (II)	Fatty, beef fat	33	6	1095	1094	1441	1434	MS, LRI
Nonanal	Floral, fruity	12	3	1103	1107	1387	1375	O, MS, LRI

Compound	Odour description[a]	Intensity[b]	Detection frequency[c]	LRI_{DB-5}[d]		LRI_{ZB-Wax}[e]		ID[f]
				GC-O	GC-MS	GC-O	GC-MS	
2-Acetyl-2-thiazoline	Toasted	26	4	1109	1111	n.d.	n.d.	O, MS, LRI
4-Methyl-2-isopropylthiazole	Roasting tin bits, pyrazine, seasoning, onions, soil	22	4	1116	1120	-	-	O, MS
(Z)-2-Nonenal	Fatty, sheets	24	4	1149	n.d.	1498	1489	O, ms, lri
(E,E)-2,6-Nonadienal	Violets, floral, waxy, fatty	28.5	6	1155	n.d.	1580	n.d.	O, LRI
(E)-2-Nonenal	Fatty, plastic-like	29	6	1162	1165	1528	1529	O, MS, LRI
(E,E)-2,4-Decadienal	McCains	5	1	1337	1327	1812	1814	O, MS, LRI
(E)-2-Tridecenal	Raw onion, herby	18	4	1372	1370	1650	n.d.	O, ms, lri
Bis(2-methyl-3-furyl)disulfide	Beef fat, meaty, fatty	5	1	1547	1550	n.d.	n.d.	O, MS, LRI

[a] Odour descriptors provided by 3 trained panellists
[b] Sum of odour intensities of duplicate samples recorded by panellists on DB-5 column (maximum score = 60)
[c] Number of times the odour was detected by panellists (maximum n = 6)
[d] Linear retention indices determined on DB-5 column (n.d. = not detected)
[e] Linear retention indices determined on ZB-WAX column (n.d. = not detected)
[f] Confirmation of identity where O = odour description agrees with literature; MS = mass spectrum agrees with that of authentic compounds; ms = mass spectrum agrees with reference spectrum in NIST 14/ Inramass MS database or in literature; LRI = linear retention indices on DB-5 and/or ZB-WAX columns (where applicable) agree with that of authentic compounds; lri = linear retention indices on DB-5 and/or ZB-WAX columns (where applicable) agree with literature values

Table 2-3: Odour active compounds in the boiled chicken (BC) extract

Compound	Odour description[a]	Intensity[b]	Detection frequency[c]	LRI_{DB-5}[d]		LRI_{ZB-Wax}[e]		ID[f]
				GC-O	GC-MS	GC-O	GC-MS	
Hydrogen sulfide	Poo, rotting chicken, rotten egg	28	6	<500	<500	<800	n.d	O, ms
Acetaldehyde	Sweet solvent	15	3	<500	<500	<800	695	O, ms, lri
Methanethiol	Old cold chicken remnants, rotting, cabbage	24	5	<500	<500	<800	n.d	O, ms, lri
Ethanethiol	Town gas, sulfurous	12	2	<500	n.d	<800	n.d	O, lri
2-Propanethiol	Onion, solvent, sulfurous	43	6	<500	<500	<800	n.d	O, ms, lri
2,3-Butanedione	Buttery, creamy	21	5	586	n.d	985	980	O, MS, LRI
1-Propanethiol	Onion, cabbage	19	6	603	608	n.d.	845	O, MS, LRI
3-Methylbutanal	Malty, cocoa, nutty	29	6	644	646	925	931	O, MS, LRI
2-Methylbutanal	Chocolate liquor, aldehydic	13	3	652	656	915	925	O, MS, LRI
Unkown	Onion, soil	10	3	654	-	-	-	-
2,3-Pentanedione	Buttery, creamy, nutty, cheesy	16	5	695	697	1058	1080	O, MS, LRI
Unknown	Biscuit, pastry, onion, cat's pee, cough syrup	16	4	735	-	-	-	-
Dimethyl disulfide	Pungent, raw, rotting onion, bad egg	18	4	744	744	1081	1083	O, MS, LRI
1-(Methylthio)propane	Rotting onions, sweaty, sour, petroleum	43	6	765	764	1256	1259	O, MS
Hexanal	Fresh grass, green	32	6	799	801	1070	1101	O, MS, LRI
Mercaptopropanone	Savoury, cooked vegetable, onion	22	4	805	n.d	1353	1351	O, ms, lri
3-Mercapto-2-butanone	Onion, chicken, meaty, sulfurous	17	4	816	818	1265	n.d	O, MS, LRI
3-Methyl-2-butene-1-thiol	Beer headspace, weed	26	5	823	n.d	n.d	n.d	O, lri
1-(Methylthio)ethanethiol + Ethyl methyl disulfide	Raw onion, catty, petroleum, crispy chicken skin	40	6	846 846	847 848	1222 1148	1231 n.d.	O, MS, LRI O, ms, lri
2-Methyl-3-furanthiol	Cooked meat, roasted, porridge oats	32	5	869	874	1330	1320	O, MS, LRI
2-Methyl-2-thiazoline	Raw onion, catty, petroleum, crispy chicken skin	45	6	877	884	1278	1284	O, MS, LRI
2,4-Dimethylthiazole	Meaty, grilled chicken, roasted	14	4	883	889	1260	1276	O, MS, LRI
(Z)-4-Heptenal	Lamb fat, potato, grilled chicken	38	6	898	900	1240	n.d.	O, MS, LRI
Heptanal	Fruity	5	1	899	903	1183	1189	O, MS, LRI

Compound	Odour description[a]	Intensity[b]	Detection frequency[c]	LRI_{DB-5}[d]		LRI_{ZB-Wax}[e]		ID[f]
				GC-O	GC-MS	GC-O	GC-MS	
3-Mercapto-2-pentanone	Sweaty, onion, cat's pee, grilled chicken	25	4	900	903	1353	1358	O, MS, LRI
Methional	Potato, fried chips	33	5	905	909	1443	1448	O, MS, LRI
2-Furylmethanethiol + 2-Mercapto-3-pentanone	Roasted, burnt, onion, meaty	34	5	909 909	912 n.d	1438 n.d.	1431 n.d.	O, MS, LRI O, lri
2,4-Dimethyl-3-thiazoline	Meaty, brothy	27	4	916	916	1289	1301	O, MS, LRI
2-Acetyl-1-pyrroline	Basmati rice, pandan	39	6	920	924	1321	1323	O, ms, lri
2,4-Dimethyl-5-ethyloxazole	Roasted/grilled meat	24	5	928	928	n.d.	n.d.	O, ms, lri
4-Mercapto-4-methyl-2-pentanone	Boiled chicken	7	2	938	936	1385	n.d.	O, ms, lri
Heptanol	Mushroom, fusel	17	3	973	973	1455	1459	O, MS, LRI
Dimethyl trisulfide	Pickled onion	9	2	977	976	1352	1351	O, MS, LRI
1-Octen-3-one	Cooked mushroom	13	2	976	983	1298	1295	O, MS, LRI
1-Octen-3-ol	Cooked mushroom	13	2	976	983	1455	1457	O, MS, LRI
(Z)-1,5-Octadien-3-one	Raw mushroom, earthy, plant, geranium	19	4	981	n.d.	1361	n.d.	O, lri
Trimethyl-3-thiazoline (I)	Roasted onion, earthy, sweaty	17	3	990	990	1364	1351	O, MS, LRI
Trimethyl-3-thiazoline (II)	Roasted onion, earthy, catty	27	5	993	999	1376	1367	O, MS, LRI
Octanal	Fruity, sweet	11	2	1003	1005	1286	1288	O, MS, LRI
(E,E)-2,4-Heptadienal	Earthy, fatty, savoury, cooked vegetable, meat broth	5	2	1016	1016	1477	1482	O, MS, LRI
2-Acetylthiazole	Basmati rice, toasted, pandan	24	5	1020	1023	n.d.	1637	O, MS, LRI
4-Ethyl-2-methylthiazole	Popcorn, basmati, barley, biscuit, roasting skin	16	3	1047	1046	1429	1427	O, MS, LRI
Phenylacetaldehyde	Floral, perfume	20	4	1051	1052	1650	1636	O, MS, LRI
2-Acetyl-1,4,5,6-tetrahydropyridine	Roasted skin, bread	17	3	1053	1053	-	-	O, ms, lri
Trimethyl-5-hydroxy-3-thiazoline	Roasted, fatty chicken skin	14	2	1055	1060	n.d.	n.d.	ms
2-Acetylpyrrole	Rice, popcorn, toasted	17	3	1069	n.d.	1969	1970	O, MS, LRI

Compound	Odour description[a]	Intensity[b]	Detection frequency[c]	LRI_{DB-5}[d]		LRI_{ZB-Wax}[e]		ID[f]
				GC-O	GC-MS	GC-O	GC-MS	
2-Ethyl-3,5-dimethylpyrazine	Pyrazine, cardboard, jackfruit seed, soil	19	4	1078	1081	1439	1446	O, MS, LRI
5-Ethyl-2,4-dimethylthiazole	Popcorn, nutty, sweet, roasted	11	2	1080	1079	1425	1424	O, MS, LRI
2-Ethyl-4,5-dimethyl-3-thiazoline (I)	Roasted onion, rice	10	2	1088	1085	n.d.	n.d.	MS, LRI
Tetramethylpyrazine	Cardboard, pyrazine, medicinal	26	5	1092	1088	n.d.	1458	O, MS, LRI
5-Ethyl-2,4-dimethyl-3-thiazoline (II)	Fatty, grilled meat, savoury	20	3	1098	1095	1453	1451	MS, LRI Au:1446
Nonanal	Fatty, citral, leafy	14	3	1108	1107	1396	1391	O, MS, LRI
2-Acetyl-2-thiazoline	Popcorn	2	1	1110	n.d.	n.d.	n.d.	O, MS, LRI
Unknown	Earthy, soil	16	3	1120	-	-	-	-
Unknown	Popcorn	5	1	1129	-	-	-	-
2-Acetyl-3,4,5,6-tetrahydropyridine	Popcorn, rice cracker/broth, roasted	20	4	1142	1142	1583	n.d.	O, ms, lri
(E/Z)-3,5-Dimethyl-1,2,4-trithiolane	Fatty, roasted, (fried) rice	18	3	1158	1157	1582	1579	O, ms, lri
(E)-2-Nonenal	Fatty, aldehydic, waxy	15	3	1161	1165	1511	1524	O, MS, LRI
2-Methyl-3-furyl methyl disulfide	Casserole, meaty, beefy, peppery	4	1	1185	1181	1630	1649	O, MS, LRI
Decanal	Orange	6	1	n.d.	1209	1493	1490	O, MS, LRI
5,6-Dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine (Thialdine)	Black onion, soil	17	4	1220	1221	1737	1736	O, ms, lri
Furfuryl methyl disulfide	Chicken, fatty	5	1	1228	1226	n.d.	1799	O, MS, LRI
+ (E,E)-2,4-Nonadienal				1228	n.d.	1699	1690	O, MS, LRI
Benzothiazole	Savoury, chicken fat, nutty	18	4	1244	1249	1944	1937	O, MS, LRI
(E/Z)-3-Ethyl-5-methyl-1,2,4-trithiolane	Blackcurrant, cat's pee, crispy chicken skin	10	2	1262	1267	1699	1687	ms, lri
Nonanoic acid	Sweaty	9	2	1267	1268	n.d.	2179	O, MS, LRI
(E,E)-2,4-Decadienal	McCains, fried, fatty, meaty	9	2	1331	1325	1814	1802	O, MS, LRI

[a] Odour descriptors provided by 3 trained panellists
[b] Sum of odour intensities of duplicate samples recorded by panellists on DB-5 column (maximum score = 60)

[c] Number of times the odour was detected by panellists (maximum n = 6)
[d] Linear retention indices determined on DB-5 column (n.d. = not detected)
[e] Linear retention indices determined on ZB-WAX column (n.d. = not detected)
[f] Confirmation of identity where O = odour description agrees with literature; MS = mass spectrum agrees with that of authentic compounds; ms = mass spectrum agrees with reference spectrum in NIST 14/ Inramass MS database or in literature; LRI = linear retention indices on DB-5 and/or ZB-WAX columns (where applicable) agree with that of authentic compounds; lri = linear retention indices on DB-5 and/or ZB-WAX columns (where applicable) agree with literature values

Table 2-4: Odour thresholds of selected alkenals and alkadienals

Alkenal/ Alkadienal	Odour threshold (μg kg^{-1} in water)	Source
(E)-2-heptenal	13	c
(E,E)-2,4-heptadienal	0.032	a
(E,E)-2,4-nonadienal	0.06	b
(E,Z)-2,6-nonadienal	0.0045 – 0.02	a, b
(E,E)-2,4-decadienal	0.027 – 0.07	a, c

[a] Flaig et al. (2020) [b] Milo and Grosch (1993) [c] Buttery et al. (1988)

Table 2-5: Mass spectral data for 5-ethyl-2,4-dimethyl-3-thiazoline

Compound	LRI_{DB-5}[a]	LRI_{ZB-Wax}[b]	Mass spectral data, m/z (relative intensity)[c]
5-Ethyl-2,4-dimethyl-3-thiazoline (I)	1080	1419	69, 42 (72), 102 (64), 73 (44), **143** (43), 41 (38), 68 (36), 45 (33), 60 (32), 114 (27)
5-Ethyl-2,4-dimethyl-3-thiazoline (II)[†]	1099	1446	69, 42 (70), 102 (58), 73 (40), **143** (40), 41 (39), 68 (34), 45 (34), 60 (30), 114 (25)

[†] Compound identified in boiled beef and boiled chicken aroma extracts [a] Linear retention index on DB-5 column [b] Linear retention index on ZB-Wax column [c] First number is the base peak; molecular ion in bold type.

2.6.2 Figures

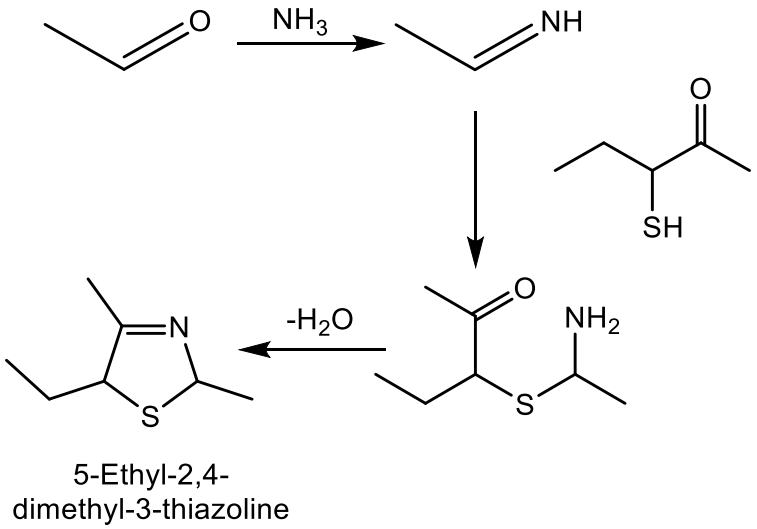


Figure 2-1: Proposed scheme for the formation of 5-ethyl-2,4-dimethyl-3-thiazoline

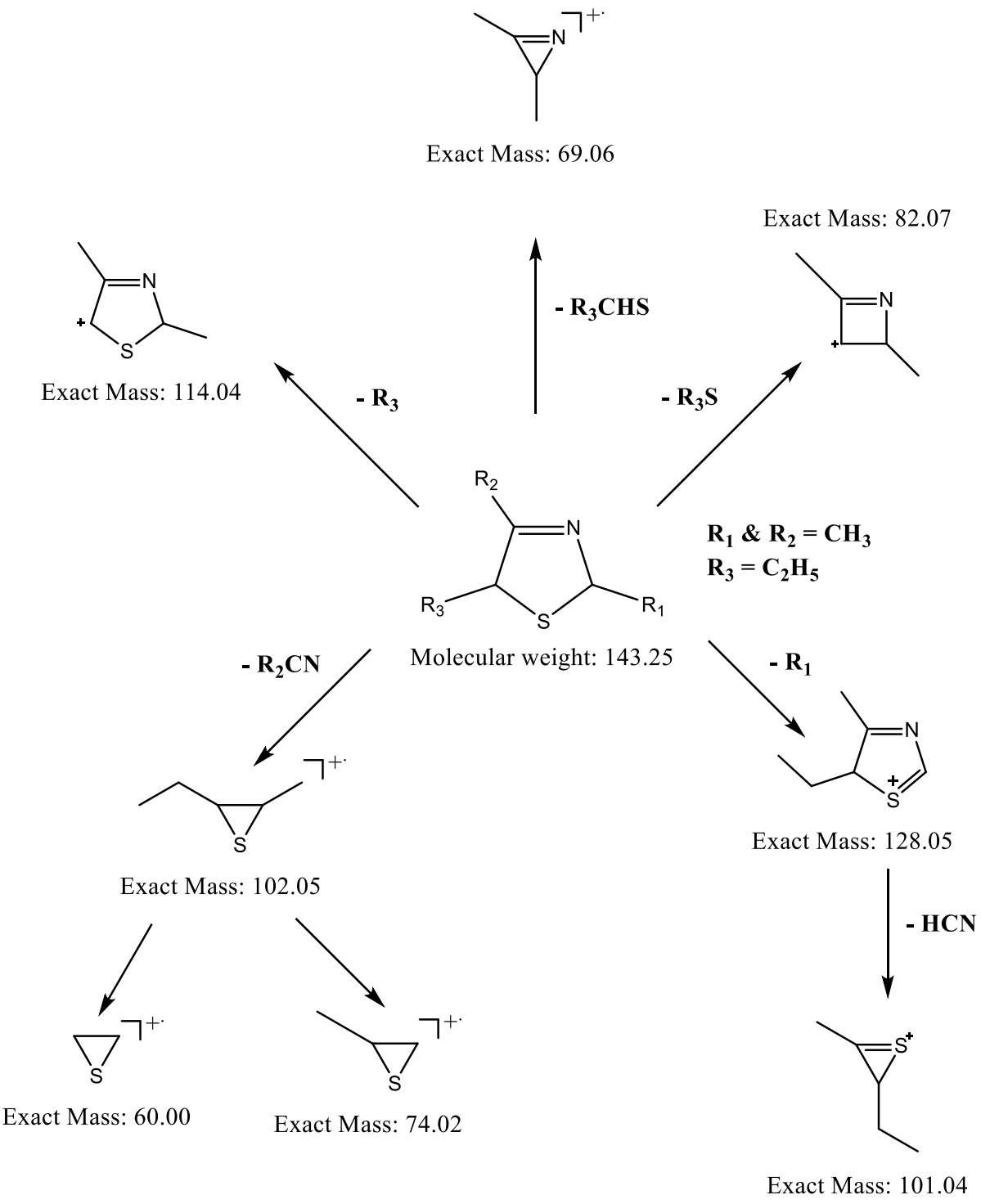


Figure 2-2: Proposed mass fragmentation pattern of 5-ethyl-2,4-dimethylthiazoline

3. Effect of triglycerides and phospholipids on boiled chicken aroma generation

Abstract

Lipids are important precursors for the formation of species-specific meat aromas. Polar phospholipids, in particular, have been shown to play a bigger role in the thermal generation of lipid-derived meat aromas than neutral triglycerides. Chicken triglycerides (TG^C) and phospholipids (PL^C) were added individually or as a mixture (1:1 w/w) to a defatted chicken meat matrix and the aroma generated during heating was extracted using Likens-Nickerson simultaneous distillation-extraction for analysis by GC-MS. The results showed that the incorporation of PL^C significantly enhanced the production of some S- and/or N-containing Maillard reaction products and most of the lipid-derived products. In addition, the extent of the individual roles of both lipids in the reaction was investigated by heating the samples at 100 °C for different durations (30, 60, 90, 120 and 150 min) and the key headspace volatiles were analysed. The results demonstrated that PL^C were more efficient in generating lipid-derived volatiles and could have a catalytic effect on the formation of key volatiles from TG^C.

Keywords: Triglyceride, phospholipid, chicken aroma, Likens-Nickerson simultaneous distillation-extraction, SPME

3.1 Introduction

In meat, muscle lipids are composed of neutral lipids, which are primarily comprised of triglycerides (TGs) in the adipocytes located along the muscle fibres and in the interfascicular area, as well as polar lipids, which mainly consist of structural phospholipids (PLs) located in the cell membranes (Raes & De Smet, 2009). The structure of TGs is relatively simple compared to other classes of lipids, consisting of 3 fatty acids (FAs) each attached to a glycerol backbone via an ester linkage. On the other hand, the structural complexity of PLs is manifested in the variety of chemical groups attached to a backbone comprised of either a glycerol, which is trihydroxy sugar alcohol, or a sphingosine, which is a long chain amino alcohol (Lehninger et al., 2012). In terms of function, while phospholipids provide essential structural integrity to cell membranes, triglycerides serve as a vital source of reserve energy for the living organism (De Carvalho & Caramujo, 2018).

The important role of lipid oxidation in meat aroma generation and off-flavour development has been well-established. The focus of the earlier studies was on the negative flavour impact of lipid oxidation. PL was identified to be the greater culprit of meat rancidity as compared to TG during the frozen storage of model systems made up of muscle fibres (Igene et al., 1980). Moreover, phosphatidylethanolamine (PE), rather than phosphatidylcholine (PC), was found to be a major contributor to warmed-over flavour while TG only enhanced the effects in the presence of PL (Igene & Pearson, 1979).

On the other hand, the research of Mottram and Edwards (1983) took a different direction and led to the revelation of the value of lipids, particularly PL, in the formation of the desirable meat aroma. They reported that the removal of TGs from freeze-dried and reconstituted lean beef extracts had little effect on the aroma of cooked meat but the removal of both TGs and PLs resulted in the substitution of the meaty aroma by generic roasted notes, which was

attributed to an increase in pyrazine formation and decrease in aliphatic lipid-derived aldehydes. Since pyrazines are characteristic Maillard reaction products, it was proposed that lipids or their degradation products acted as inhibitors of the formation of heterocyclic products during the reaction. This was later validated in model systems, in which reductions in heterocyclic Maillard reaction products were also observed upon lipid addition (Farmer and Mottram, 1990, Whitfield et al., 1988). The fact that Farmer and Mottram (1990) reported a more pronounced effect for PLs than TGs further highlighted the importance of PLs in aroma development.

The aim of the study is to investigate the role of TGs and PLs in boiled chicken (BC) aroma generation using a defatted meat matrix with the addition of lipids to achieve a system as close to real meat as possible to mimic the cooking of stocks and stews in a kitchen setting by employing Likens-Nickerson simultaneous distillation-extraction (LN-SDE). This knowledge will increase an understanding of the chemical effect of TGs and PLs on the generation of desirable cooked chicken aroma. The hypothesis is that PLs are more reactive than TGs due to their higher concentration of unsaturated FAs and thus, have a greater influence on BC aroma and can even have a catalytic effect on TGs.

3.2 Materials and methods

3.2.1 Materials and chemicals

Aroma chemicals were obtained from the following suppliers and were ≥95% in purity unless stated otherwise: hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, tridecanal, 3-(methylthio)propanal, 2-methylbutanal, (E)-2-octenal, (E)-2-nonenal, (E)-2-hexenal, 1-octanol, 1-nonanol, 1-octen-3-ol, (E)-2-octen-1-ol, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, 2-undecanone, 1-octen-3-one, (E)-3-nonen-2-one, 2,3-pentanedione, 2,3-octanedione, dimethyl disulfide, dimethyl trisulfide, benzothiazole, tetramethylpyrazine

and 2-isopropylpyrazine from Sigma Aldrich (Gillingham, UK); 2-ethyl-3,5-dimethylpyrazine from Fluorochem (Hadfield, UK); 3-methylbutanal from Alfa Aeser (Heysham, UK); 2-phenylacetaldehyde from Acros Organics (New Jersey, USA); 2-pentylfuran from Avocado Research Chemicals (London, UK); (E)-2-decenal and (E)-2-heptenal from Fluka (Seelze, Germany); (E,E)-2,4-decadienal (90%) from Lancaster Synthesis (Heysham, UK); 1-hexanol from IFF (Haverhill, UK); 1-pentanol, 4-nonanone from TCI (Oxford, UK) and (E,E)-2,4-decadienal-d4 from aromaLAB GmbH (Martinsried, Germany).

Diethyl ether, pentane (98%), petroleum ether (boiling point 40 – 60 °C; ≥75%), isooctane, 0.5 M sodium methoxide solution in methanol (0.5 N), sodium chloride, methyl tricosanoate, Supelco 37 Component FAME Mix (CRM47885), amino acid standard (AAS18), L-norvaline, D-(-)-ribose, D-(-)-fructose, D-(+)-mannose, D-(+)-glucose, L-rhamnose, D-ribose-5-phosphate disodium salt hydrate (≥98%), D-fructose-6-phosphate disodium salt hydrate (≥98%), D-mannose-6-phosphate disodium salt hydrate (≥97%), D-glucose-6-phosphate disodium salt hydrate (≥98%), anhydrous dimethyl sulfoxide, hexamethyldisilazane, trimethylchlorosilane and anhydrous cyclohexane were from Sigma-Aldrich. Chloroform, methanol, acetonitrile, sodium chloride and ammonium formate were from Fisher Scientific (Loughborough, UK). Formic acid and Florisil® (30 – 60 mesh) were from VWR Chemicals (Lutterworth, UK). All the chemicals were ≥99% in purity unless specified otherwise. All the chemicals used for LC-MS analysis were of LC-MS grade. Medium chain triglycerides (Ester 610) were obtained from Chempri BV Oleochemicals (Raamsdonkveer, The Netherlands). High purity water (18.2 MΩ) was obtained from a Select Fusion Ultrapure water deionisation unit (SUEZ, Peterborough, UK) for instrumental analysis while filtered tap water was used for sensory evaluation.

3.2.2 Chicken processing

Fresh Class A chicken breasts were purchased from a retail supermarket and processed within the sell-by date. They were a standard Ross 308 genotype provided by one commercial poultry supplier and were from the same batch within an experiment. The chicken breasts were trimmed of extramuscular fat and minced using a food mincer with a 4.5 mm screen (Kenwood, Hampshire, UK). A portion of the minced meat was vacuum packed in aluminium pouches and stored at -80 °C. Another portion was freeze-dried, ground and thoroughly mixed before storage in the same manner.

3.2.3 Chicken lipid extraction and purification

A 2-stage Soxhlet lipid extraction was carried out on freeze-dried meat: first, using petroleum ether followed by chloroform:methanol (2:1 v/v) to obtain a chicken triglyceride (TG^C) and chicken phospholipid (PL^C) fraction respectively. Both stages were carried out at 50 – 60 °C for 8 and 6 cycles respectively before the solvent was removed by rotary evaporation at 40 °C. The lipid extracts and meat fraction were kept under vacuum overnight to remove residual solvent. The TG^C extract was purified using Florisil® following the procedure of Farmer and Mottram (1990) while the PL^C extract was washed using 0.2 times the volume of Ultrapure water (Folch et al., 1957) before dissolution in chloroform for storage. All lipid extracts were stored under nitrogen at -20 °C while the defatted meat was vacuum packed in aluminium pouches and stored at -80 °C.

The lipid extracts were tested for contamination using thin-layer chromatography (TLC) on TLC silica gel 60 F_{254} plates (Supelco, Merck Millipore, Gillingham, UK) eluted with hexane: diethyl ether:formic acid (80:20:2 v/v). Different amounts (10 and 50 μg) of each lipid were applied to the TLC plate to estimate the level of impurities. The spots were visualised using a phosphorous spray (Appendix A). As the purpose of the purification procedure was to obtain

a lipid sample in sufficient quantities for characterisation and experiments, and not to obtain an accurate lipid compositional analysis for the muscle, this degree of separation was considered adequate.

3.2.4 Fatty acid analysis

Fatty acid methyl ester (FAME) derivatisation was carried out using an adapted method from Bannon et al. (1982). Briefly, 1 mL of diethyl ether containing 1 mg mL^{-1} methyl tricosanoate as internal standard and 2 mL of 0.5 M sodium methoxide solution in methanol were added to 50 mg of lipid before stirring for 5 min at 1000 rpm. This was followed by the addition of 2 mL of isooctane and 5 mL of saturated sodium chloride solution before vigorously shaking at 1800 rpm. Upon separation, the upper layer was removed for analysis.

FAME separation was performed on an Agilent 7890B GC coupled to a flame ionisation detector (Agilent Technologies, Santa Clara, CA, USA) using a HP-88 column (100 m x 0.25 mm x 0.2 μm; Agilent Technologies). The injection volume was 1 μL at a 50:1 split ratio. The oven temperature was increased from 120 °C (held for 1 min) to 175 °C at 10 °C min^{-1} (held for 10 min), then to 210 °C at 5 °C min^{-1} (held for 5 min), and finally to 230 °C at 5 °C min^{-1} (held for 5 min). The carrier gas was hydrogen at a constant flow rate of 1.5 mL min^{-1}. The temperature of the injector and detector were 250 °C and 280 °C respectively. Individual FAMEs were identified by comparing their retention times with those of a standard 37 component FAME mix. Quantification of the compounds was performed by comparison of their respective peak areas against that of the internal standard. A total of 3 replicates were carried out and the results were expressed as percentage of total FAs (%).

3.2.5 Free sugar and sugar phosphate analysis

An adapted method from Koutsidis et al. (2008) was used. Freeze-dried (0.5 g) and defatted (1 g) meat were extracted with 10 mL of deionised water. The sample was shaken for 5 min

followed by centrifugation at 10,000 *g* at 4 °C for 10 min. The supernatant was decanted while the residue was re-extracted twice as previous and the supernatants were combined. Rhamnose (40 ng μL^{-1} in water) was used as the internal standard. Aliquots of the aqueous extracts (0.5 mL) were freeze-dried in reaction vials (Pierce & Warriner, Chester, UK). For the derivatisation, 1 mL of anhydrous dimethyl sulfoxide, 0.2 mL of hexamethyldisilazane, 0.1 mL trimethylchlorosilane and 1 mL anhydrous cyclohexane were added. The samples were shaken at 1800 rpm for 5 min to ensure the complete dissolution of the freeze-dried residues before sonication for 30 min. The vials were left to stand in a 25 °C water bath for 24 h and the upper layer was removed for analysis.

The analyses were performed on an Agilent 7890A GC (Agilent Technologies) equipped with an Agilent 5975C inert XL EI/CI triple axis mass spectroscopy detector (Agilent Technologies). An aliquot of sample (1 μL) was injected in splitless mode. Chromatographic separation was carried out on a DB-17 MS column (30 m x 0.25 mm x 0.25 μm; Agilent Technologies) coupled to a 1.5 m of deactivated methylsilicone fused silica capillary as a retention gap. The oven temperature was increased from 60 °C (held for 1 min) to 130 °C at 30 °C min^{-1} (held for 2 min), then to 170 °C at 2 °C min^{-1} and finally to 300 °C at 4 °C min^{-1}. The carrier gas was helium at a constant flow of 1 mL min^{-1}. The MS was operated in electron impact mode with a source temperature of 300 °C, ionisation energy of 70 eV and a scan range from m/z 20 to m/z 400. Calibration curves were built between 5 – 1250 μmol mL^{-1}. A total of 3 replicates were carried out and the results were expressed as mg 100 g^{-1} wet weight by taking into account the 75% moisture present in chicken breast.

3.2.6 Free amino acid analysis

The extraction was carried out as above in Section 3.2.5. Norvaline (30 ng μL^{-1} in water) was used as the internal standard. The combined supernatants were diluted with acetonitrile in a

1:4 v/v ratio before centrifugation at 12,100 *g* for 10 min and filtration through a 0.2 µm PTFE syringe filter (Fisher Scientific). The analyses were performed on an Agilent 1260 Infinity HPLC system coupled to an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies) equipped with a standard micro-flow source. Chromatographic separation was carried out on a Synchronis HILIC column (150 × 4.6 mm, 3 µm; Thermo Fisher Scientific, Loughborough, UK) with a Synchronis HILIC precolumn (10 × 4.6 mm, 3 µm; Thermo Fisher Scientific) maintained at 40°C. The injection volume was 5 µL. Mobile phases A and B consisted of 5 mM ammonium formate with 0.5 % formic acid in water and acetonitrile:water (9:1 v/v) respectively. The column flow rate was set at 1 mL min^{-1} using the following gradient elution: 10 % to 40 % A in 8 min and decreased to 10 % A in 1 min (held for 4 min). The electrospray ionisation source was set at a gas temperature of 330 °C, with a gas flow of 13 L min^{-1}, nebuliser pressure of 40 psi, cell acceleration voltage of 7 V, using the dynamic multiple reaction monitoring (MRM) scan mode under the conditions in Appendix B. Optimised source and fragmentation parameters were selected independently for all transitions by monitoring MS responses over a broad range and noting maximum signal intensity. Calibration curves were built between 0.5 - 250 µmol mL^{-1}. A total of 4 replicates were carried out and the results were expressed as mg 100 g^{-1} wet weight by taking into account the 75% moisture present in chicken breast.

3.2.7 Preliminary experiment

3.2.7.1 Sample preparation

The composition of the reconstituted samples is listed in Table 3-1. Samples with lipids, sugars and amino acids added had the same composition as the TG^C- and PL^C-containing samples, with the addition of the sugars and amino acids listed in Table 3-2 to account for precursor loss during lipid extraction. An equal part of water was added to facilitate mixing and for sample heating. All the samples were prepared in 20 mL glass vials, flushed under a gentle

stream of nitrogen for 5 min, sealed with the vial lid and parafilm, mixed using a vortex mixer and stored at 4 °C overnight for equilibration. The internal standard was 4-nonanone (1 ng μL^{-1} in methanol) and added the following day before the samples were heated in a 100 °C water bath for 30 min. A total of 4 replicates were prepared for each treatment (FD = Freeze-dried meat sample; DF = Defatted meat sample containing MCT; CTG = Defatted meat sample containing TG^C; CTGPL = Defatted meat sample containing TG^C and PL^C (1:1 w/w); CPL = Defatted meat sample containing PL^C; LSA = Defatted meat sample containing TG^C and PL^C (1:1 w/w) with the addition of sugar and amino acids).

3.2.7.2 Headspace-Solid Phase Micro-Extraction Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS)

The samples were incubated at 60 °C for 5 min before extraction at the same temperature for 20 min using a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (Supelco, Bellefonte, PA, USA). The fibre was then desorbed in the injection port at 250 °C for 20 min. GC-MS analyses were performed on an Agilent 7890A GC (Agilent Technologies) equipped with an Agilent 5975C inert XL EI/CI triple axis mass spectroscopy detector (Agilent Technologies). Chromatographic separation was carried out on a Zebron ZB-5MSi column (30 m x 0.25 mm x 1 μm; Phenomenex, CA, USA). The oven temperature was increased from 35 °C (held for 10 min) to 200 °C at 6 °C min^{-1} and to 320 °C at 15 °C min^{-1} (held for 10 min). The carrier gas was helium at a constant flow rate of 1 mL min^{-1}. The MS was operated in electron impact mode with a source temperature of 230 °C, ionisation energy of 70 eV and a scan range from m/z 20 to m/z 300.

A series of C_5 – C_{25} *n*-alkanes was analysed under the same conditions for the calculation of the linear retention index (LRI) of each compound. The identities of the compounds were confirmed based on a match of their mass spectra and LRI with those of authentic compounds

where available. Otherwise, a tentative identification was made by comparing their mass spectra against the NIST 14 library and LRI available in literature. Semi-quantification of the compounds was performed by comparison of their respective peak areas against that of the internal standard using relative response factors (RRFs). The quantification ions and RRFs used are listed in Appendix C.

3.2.7.3 Sensory evaluation

The aroma of the samples was assessed by 10 trained panellists using free-choice profiling (FCP). The samples were reconstituted in 20 mL amber glass vials labelled with random 3-digit codes as described for SPME analysis in 3.2.7.2. On the day of the evaluation, the samples were heated in one batch in an electric steamer (Tefal UltraCompact VC145140, Tefal, Datchet, UK) for 30 min and transferred to a tray of hot sand to be kept warm before presentation to the panellists in a random and balanced order. Upon receiving the sample, the panellists were instructed to swirl the vial in small circular motions before removing the lid for aroma evaluation. During the attribute generation session, each panellist created their unique list of descriptors and scale anchors, which were used for the subsequent evaluation of the samples using a 100-point unstructured line scale. All the samples were assessed in duplicates during separate scoring sessions on different days by the panellists in ventilated sensory booths illuminated with white light.

3.2.8 Likens-Nickerson simultaneous distillation-extraction experiment

3.2.8.1 Sample preparation

Frozen minced meat (FMM) samples were allowed to defrost overnight in the aluminium pouches at 4 °C. The composition of the reconstituted samples is listed in Table 3-3. An equal part of water was added to facilitate mixing and for sample heating. All the samples were prepared in 1 L round bottom flasks and the reconstituted samples were flushed under

nitrogen for 5 min, stoppered and sealed with parafilm, mixed using a magnetic stirrer until the lipid was incorporated and stored at 4 °C overnight for equilibration. An internal standard mix of 2-isopropylpyrazine and 4-nonanone (both 10 ng μL^{-1} in 100 μL methanol) was added to the sample the following day before extraction. A total of 4 replicates were prepared for each treatment.

3.2.8.2 Likens-Nickerson simultaneous distillation-extraction

Each 25 g sample was boiled at 100 °C in a heating mantle for 30 min. LN-SDE was performed using 30 mL of redistilled pentane:diethyl ether (9:1 v/v) for 2 h. The extract was concentrated to 0.5 mL using a Vigreux column, flushed under a gentle stream of N_2 to 0.1 mL and stored at -80 °C prior to analysis.

3.2.8.3 GC-MS analysis of aroma extracts

GC-MS analyses were performed on an Agilent 6890N GC equipped with an Agilent 5975 inert XL EI/CI triple axis mass spectroscopy detector (Agilent Technologies, Santa Clara, CA, USA). An aliquot of sample (1 µL) was injected in a pulsed splitless mode (pulse pressure18.5 psi; 0.5 min). Chromatographic separation was carried out on a HP-5 MS column (30 m x 0.25 mm x 1 μm, Agilent Technologies). The oven temperature was increased from 35 °C (held for 10 min) to 200 °C at 6 °C min^{-1} and to 320 °C at 15 °C min^{-1} (held for 10 min). The carrier gas was helium at a constant flow rate of 1.2 mL min^{-1}. The MS was operated in electron impact mode with a source temperature of 230 °C, ionisation energy of 70 eV and a scan range from m/z 20 to m/z 400. Compound identification and semi-quantification were as described in Section 3.2.7.2 except that 2-isopropylpyrazine was the internal standard for S- and/or N-containing compounds while 4-nonanone was used for the other compounds. The quantification ions and RRFs used are listed in Appendix D.

3.2.9 Kinetics experiment

3.2.9.1 Sample preparation

CTG, CPL and CTGPL samples were reconstituted as in Section 3.2.7.1 except that the samples were mixed using a magnetic stirrer for 10 min at 990 rpm and the vials were left open for 1 min to allow oxygen to enter the headspace prior to heat treatment. The samples were heated in a 100 °C water bath for a duration of 30, 60, 90, 120 and 150 min. An internal standard mix of 4-nonane and deuterium-labelled (E,E)-2,4-decadienal-d4 (100 ng μL^{-1} and 500 ng μL^{-1} respectively in 5 μL methanol) was immediately added to the sample using a syringe injected through the PTFE/silicone-lined septum and placed on the machine for SPME analysis without delay. A total of 4 replicates were prepared for each treatment.

3.2.9.2 HS-SPME-GC-MS

The same conditions were used as in Section 3.2.7.2 with the following changes. The oven temperature was increased from 40 °C (held for 2 min) to 200 °C at 6 °C min^{-1} and to 320 °C at 15 °C min^{-1} (held for 10 min). A full scan alongside a SIM acquisition of the major/key ion and molecular weight ion of labelled (E,E)-2,4-decadienal-d4 and non-labelled (E,E)-2,4-decadienal (*m/z* 81, *m/z* 152 and *m/z* 156) were performed. Compound identification and semi-quantification were as described in Section 3.2.7.2 except that (E,E)-2,4-decadienal was quantified using its labelled standard while 4-nonanone was used for the other compounds. The quantification ions and RRFs used are listed in Appendix E.

3.2.10 Statistical analysis

All statistical analyses were carried out using XLSTAT v2021.4.1 (Addinsoft Inc, Paris, France). Statistical differences between samples were tested using 1-way analysis of variance (ANOVA) and Fisher's least square significant difference (LSD) post-hoc test at a significance level of $p = 0.05$. The sensory data was analysed using Generalised Procrustes Analysis (GPA). Where

applicable, principal components analysis (PCA) was also performed to provide a visual representation of the data.

3.3 Results and discussion

3.3.1 Fatty acid composition of lipids

The predominant FAs in TG^C and PL^C, which were extracted from the meat samples used in the LN-SDE and kinetics experiment, were C16:0 (palmitic), C18:0 (stearic), C18:1n-9 (oleic) and C18:2n-6 (linoleic), albeit in varying proportions, and contributed to ≥ 80% of total FA content. The values in Table 3-4 were mostly in close agreement with literature data (Ferioli & Caboni, 2010; Jahan & Paterson, 2007; Marion & Woodroof, 1965; Sahasrabudhe et al., 1985). Some variations could be attributed to the effect of different production regimes and dietary supplementations on the FA profiles of chicken as reported by many studies (Cortinas et al., 2004; Givens et al., 2011; Jahan & Paterson, 2007; Wood et al., 2008). A graphical representation of the different FA classes, namely saturated fatty acid (SFA), monounsaturated fatty acid n-9 (MUFA n-9), polyunsaturated fatty acid n-3 (PUFA n-3), PUFA n-6, is provided in Figure 3-1.

In terms of the SFAs, TG^C had a 9.5% lower concentration than PL^C (29.0% v.s. 38.5%). As for the unsaturated fatty acids (UFAs), TG^C had an 18.6% higher concentration of MUFAs (43.6% v.s. 25.0%) but 8.9% lower concentration of PUFAs (27.4% v.s. 36.3%) as compared to PL^C. C18:1n-9 accounted for the majority of the MUFAs present in both lipids but was present at 16.7% higher in TG^C than PL^C (38.4% v.s. 21.7%). Meanwhile, the PUFA composition in TG^C and PL^C was predominantly made up of C18:2n-6 at 22.9% and 21.8% respectively, but there was more variation in that of PL^C with the presence of long chain FAs (≥C20) with ≥2 C=C double bonds, such as C20:4n-6 (arachidonic), C20:5n-3 (eicosapentaenoic) and C22:6n-3 (docosahexaenoic). These differences were also reflected in

the ratio of n-3, n-6 and n-9 FAs in the lipids. While TG^C had a 15% higher proportion of n-9 FAs owing to the higher concentration of C18:1n-9, PL^C had a 8% higher distribution of n-6 FAs as a result of the additional contribution from C20 FAs with various C=C double bonds, namely C20:2n-6 (eicosadienoic), C20:3n-6 (eicosatrienoic) and C20:4n-6 at 1.1%, 1.4% and 7.6% respectively, which were present in very low quantities in TG^C. Although the n-3 FAs were present within the same range of 3 – 4% in both lipids, PL^C was composed of an overall significantly higher concentration than TG^C, owing to the presence of n-3 FAs with longer carbon chains (≥20) and more sites of unsaturation (≥2), such as C20:5n-3 and C22:6n-3 at 1.02% and 0.83% respectively, as compared to C18:3n-3, which was the predominant n-3 FA present at 3.07% in TG^C but only at 1.69% in PL^C.

These differences could be attributed to the biological importance of PLs as integral constituents of the cell membrane. The presence of double bonds in the FA structure provides the carbon chain mobility and the existence of highly polyenoic FAs in turn confers fluidity and flexibility on the cell membrane for the retention of its physical properties and integrity of cellular functions at physiological temperature (Brash, 2001). Hence, a homeostatic mechanism exists to maintain a balanced ratio of SFAs to UFAs in PLs within a relatively narrow range for the maintenance of the physical state the cell membrane and performance of metabolic activities (Villaverde et al., 2006).

3.3.2 Free sugar, sugar phosphate and amino acid composition of chicken

Water-soluble compounds, such as free sugars and amino acids, which exist naturally or develop post-slaughter, serve as the precursors for the Maillard reaction (Macy et al., 1964). The values for freeze-dried meat in Table 3-5 and Table 3-6 were within the range of literature data for sugars and sugar phosphates (Aliani & Farmer, 2002; Fan et al., 2018; Koutsidis, 2004) and amino acids (Aliani & Farmer, 2005; Koutsidis, 2004) respectively. However, the

literature values are wide-ranging albeit unavoidable as various factors, ranging from pre-slaughter to post-mortem handling, could affect energy metabolism in poultry birds and consequently the pool of precursors generated (Petracci et al., 2010; Sams, 1999; Wang et al., 2017). Such animal-to-animal variation is also common in other meat species (Dashdorj et al., 2015; Khan et al., 2015).

As observed in Table 3-5, the total concentration of sugars in freeze-dried meat at 60 mg $100g^{-1}$ wet weight was almost twice that of the sugar phosphates at 34 mg 100 g^{-1} wet weight. In addition, glucose and ribose were the most and least abundant sugars with concentrations of 52.0 and 2.11 mg 100 g^{-1} wet weight respectively, whereas G6P and R5P were the most and least abundant sugar phosphate with concentrations of 24.5 and 1.02 mg 100 g^{-1} wet weight respectively. Glucose and G6P are derived from the irreversible anaerobic glycolysis, which occurs when the depletion of oxygen supply and cessation of blood circulation stimulate the breakdown of glycogen to lactic acid, yielding these compounds as intermediates (Lawrie & Ledward, 2006). Meanwhile, ribose and R5P are produced during the cascade of enzymatic reactions involved in the degradation of adenosine triphosphate (ATP), an essential ribonucleotide for muscle function (Parker, 2017). Since glycogen is an energy store and naturally present in higher abundance than ATP in the muscle, the intermediates from the glycolytic pathway would be present in higher concentrations than the products of ATP degradation. In addition, most of the potential ribose and R5P could be bound in the other forms in the degradation pathway (e.g. inosine 5'-monophosphate and inosine which are the immediate precursors to R5P and ribose). Furthermore, there was also the possibility of additional breakdown pathways for ribose and R5P which could reduce the concentrations of these precursors (Aliani & Farmer, 2002).

As for the amino acids in Table 3-6, proline was found to be present at the highest concentration at 87.4 mg 100 g^{-1} wet weight, unlike previous studies which reported glutamic acid (Koutsidis, 2004) and alanine (Aliani & Farmer, 2002) to be the most abundant .This could be due to a dietary effect whereby proline supplementation was used to enhance growth and weight gain in the poultry birds for commercial purposes (Blair et al., 1972; Graber et al., 1970) due to the inadequate synthesis of this amino acid relative to their nutritional and physiological needs (He et al., 2021). The S-containing amino acids, cysteine and methionine, which are important contributors to meat aroma formation, constituted around 5% of the total free amino acid composition. Comparing between freeze-dried and defatted meat, it was clear that the Soxhlet lipid extraction had inadvertently resulted in a significant loss of water-soluble precursors, which could occur during the second stage of extraction involving the use of polar solvents or due to their participation in the Maillard reaction under relatively milder heating conditions. There was an average of 80% loss of sugars and sugar phosphates while it was lesser at 40% for the amino acids.

3.3.3 Preliminary experiment

The purpose of the preliminary experiment was to provide a quick answer to the question of whether the addition of the precursors lost during lipid extraction was necessary. Thus, the discussion in this section would mainly be confined to the comparison between CTGPL and LSA samples while a detailed analysis between the other samples could be found in Section 3.3.4.

3.3.3.1 HS-SPME analysis

HS-SPME was selected as the method of analysis as the procedure is relatively fast and simple. As sugars and amino acids are important contributors to the Maillard reaction, changes in the quantities of these precursors could have an impact on the abundance of Maillard reaction

products formed. However, as observed from Table 3-7, there were no significant differences in the abundance of the two Maillard reaction products identified, namely 3-(methylthio)propanal and 2-phenylacetaldehyde, between CTGPL and LSA samples. In addition, there were also no significant differences in the majority of the lipid-derived compounds between these samples, except for hexanal, 1-octen-3-ol, octanal, nonanal, decanal and 2,3-octanedione. A possible explanation is that the presence of additional sugars and amino acids in the LSA samples could drive the lipid oxidation pathways to be in favour of some compounds over others although this theory needs to be explored. Meanwhile, the 'salting out' effect due to the addition of solutes is a well-known concept, whereby the active hydroxyl groups of the sugars and amino acids could form H bonds with the H atoms on the water molecules, resulting in a decrease in the volume of free water available for the solubilisation of aroma compounds (Friel et al., 2000). Thus, the effective partition equilibrium of the volatiles shifted towards the headspace, which led to the enhanced aroma release observed for some of the compounds in the LSA samples. Nevertheless, both samples were closely associated in the PCA plot (Figure 3-2), which separated the PL-containing samples from the others along the first axis while TG-containing samples were separated along the second axis. Principal components 1 (PC1) and 2 (PC2) accounted for 74.5% and 17.2% of the total variation respectively. In addition, it could also be observed from Figure 3-2 that the FD and DF samples were much further separated from the other samples. Although this was expected for the DF sample due to the absence of reactive lipids to promote lipid oxidation, the FD sample contained similar concentrations of non-volatile precursors as the LSA samples (difference being a plausibly higher TG^C:PL^C w/w ratio as compared to the equivalent weight ratios in reconstituted samples). Thus, a likely explanation would be the effect of sample processing on the meat cellular matrix, which is discussed in greater detail in Section 3.3.4.2.

3.3.3.2 Sensory evaluation

It is appreciated that the list of Maillard reaction products present in chicken aroma is much longer than the ones identified in Table 3-7 and there could be differences overlooked as a result of the lack of instrument sensitivity. Thus, FCP was used as a rapid tool for the aroma evaluation of the samples to confirm the findings obtained by HS-SPME. Figure 3-3 depicts the visual representation of the FCP data with the odour descriptors generated by the panellists provided as a means of understanding the odour differences between the samples. The first axis (F1) separated the samples containing TGPL, either naturally present or reconstituted, while the second axis (F2) separated the samples with a higher proportion of TG or sugars and amino acids. F1 and F2 accounted for 38.2% and 33.5% of the total variation within the data respectively. In addition, it could be observed that CTG samples were more associated with fatty, oily and greasy notes while CPL samples were more uniformly described to possess boiled chicken or chicken stock character, although some off-notes were also reported. FD, CTGPL and LSA samples were more complex as the panellists perceived different odour characteristics, ranging from roast to boiled chicken, as well as savoury, sweet and fatty notes. This correlated with the instrumental results (Figure 3-2) which showed that the CTGPL and LSA samples were more associated with the Maillard reaction products (e.g. 2-phenylacetaldehyde and 3-(methylthio)propanal) and lipid-derived aldehydes (e.g. (E,E)-2,4-decadienal and 1-octen-3-ol). Although the FD sample was not associated with these volatiles by instrumental analysis possibly due to the much lower abundance present, the complete profile could still be detected by the panellists, explaining the close positions of FD, CTGPL and LSA samples. As these samples were clustered together in the same region and the consensus test performed showed that the consensus configuration is a true consensus (Appendix F), it was decided that the addition of precursors lost during lipid extraction was not necessary as it would not bring about huge changes to the sample matrix.

3.3.4 LN-SDE experiment

3.3.4.1 Effect of extraction method on volatile detection and identification

The more laborious LN-SDE was employed in this experiment to obtain more concentrated aroma extracts of the meat samples for analysis by GC-MS with enhanced sensitivity and wider range of chemical classes, such as thiazoles, thiazolines and pyrazines, as observed in Table 3-8. Besides the difference in sample size and extraction duration which were altered to cater to the apparatus volume and sample size respectively, the extraction technique itself would also have an impact on the types and quantities of volatiles generated.

In HS-SPME, volatile extraction is dependent on the equilibrium between the sample, headspace and the fibre. Thus, volatiles which were present in low abundance or had a higher affinity for the matrix than the headspace would have smaller quantities partitioned into the headspace and in turn, adsorption onto the fibre. More specifically, the challenge encountered with the use of HS-SPME in the preliminary experiment was the difficulty in the detection of very low abundance of S- and/or N-containing volatiles derived from the Maillard reaction, resulting in the under-representation of these classes of compounds as observed in Table 3-7. On the other hand, there could be an over-representation of lipid-derived compounds, such as the more hydrophobic long-chain aldehydes, which partition more readily into the headspace from the aqueous phase and adsorb onto the fibre. However, high molecular weight compounds tend to have low volatilities and high odour detection thresholds, rendering their contribution to meat aroma less significant.

Meanwhile in LN-SDE, the combination of steam distillation with solvent extraction in the apparatus involves the transfer of volatile compounds in the steam to the solvent during heating before both liquids condense and return to their starting vessels. The in-situ heating of the sample facilitates the capture of aroma intermediates during the heating process,

thereby mimicking the cooking of meat in stocks and stews in a realistic kitchen setting. However, a frequently cited drawback of LN-SDE is artifact formation due to contamination, oxidation or thermal reactions (Chaintreau, 2001). Thus, a blank sample was analysed and it could be confirmed that the volatiles reported in Table 3-8 were not found in the blank.

3.3.4.2 Effect of processing on volatile generation

It could be observed from Table 3-8 that the abundance of volatiles generally increased with the extent of processing of the samples, ie. FMM < FD < DF < DF with added lipids, which could be attributed to the inevitable alteration of the cellular structure and constituents during the processing steps.

Firstly, the cutting and mincing involved at the beginning could cause disintegration of lyosomal membranes in muscle tissues, resulting in the cellular release of lipolytic enzymes and other oxidation catalysts (e.g. Fe^{2+}-containing cytochromes or haemoglobin), which could now come into contact more easily with the muscle lipids. Moreover, the mechanical disruption of muscle tissues could also induce membrane lipids to form smaller vesicles and the resultant increase in surface area could accelerate degradation (Erickson, 2002). In addition, the higher retainment of volatile hydrophobic compounds in these dispersed lipid vesicles as compared to the original structured fat in the meat cells was also plausible. Subsequently, during the freeze-drying process whereby the drying temperature is below the glass transition temperature, there would be minimal product shrinkage, leading to the formation of highly porous structures with enhanced surface area. As a consequence, an increase in diffusion of oxygen from the surface to the inner layers could occur and thus, promote oxidation as observed in FD minced beef in comparison with hot-air dried products (Aksoy et al., 2019). Finally, the use of solvents during Soxhlet extraction would have caused irreversible cell damage, which would be necessary for efficient lipid extraction. While the neutral TGs can be

extracted with non-polar solvents such as petroleum ether, polar PLs associated with cell membranes or other macromolecules, such as proteins and polysaccharides, require the use of stronger and more polar solvents such as methanol to achieve the disruption of hydrogen bonds and electrostatic forces for the successful extraction of the PLs (Pati et al., 2016; Saini et al., 2021). Although thermal treatment during cooking would also cause disruption to the cellular structure, freeze-drying and lipid extraction would be more invasive than normal kitchen processing.

Although some compounds, such as 2-methyl-3-furanthiol, 2-furylmethanethiol and 2-mercapto-3-pentanone, were not found in these samples, it was likely that they were present in extremely low quantities due to the small sample size used and could not be detected by the GC-MS. The presence of these compounds had previously been reported in chicken (Fan et al., 2018; Farkaš et al., 1997; Gasser & Grosch, 1990; Kerscher & Grosch, 1998). While it would be ideal to use a larger sample size, the extraction and purification of sufficient lipids and defatted matrix for reconstitution within a reasonable time frame to avoid sample oxidation had made it logistically challenging to accomplish. Notwithstanding the undesirable changes to the matrix due to the various forms of processing, the defatted meat was undoubtedly a system as close to real meat as possible. A potential alternative for future considerations could be myofibrillar proteins as prepared in the work of Nishimura et al. (2010).

3.3.4.3 Effect of lipid class on volatile generation

3.3.4.3.1 Lipid-derived products

Table 3-8 lists the key volatiles identified in the LN aroma extracts. Significant differences in the abundance of lipid-derived products were observed between the samples and the quantities of the majority of the lipid-derived volatiles generally increased in the following order: CTG < CTGPL < CPL samples. This trend could be observed in Figure 3-4 which

illustrates some of the lipid-derived volatiles. This could be attributed to the significantly higher composition of n-3 FAs (3.82% v.s. 3.24%) and n-6 FAs (32.5% v.s. 24.1%) present in PL^C as compared to TG^C. Moreover, a significantly higher proportion of n-3 and n-6 FAs with longer carbon chains (≥20) and more sites of unsaturation (≥2 C=C double bonds), such as C20:5n-3 (0.95%), C22:6n-3 (0.77%), C20:4n-6 (7.18%) and C22:2n-6 (0.36%) with their difference listed in brackets, would lead to increased oxidative susceptibility and higher reactivity of PL^C (Dominguez et al., 2019).

Lipid oxidation proceeds through a free radical chain reaction consisting of chain initiation, propagation, and termination. The rate-limiting step in the reaction is the abstraction of hydrogen radical from the lipid substrates to form lipid free radicals. Since hydrogen abstraction occurs at the bis-allylic positions present in PUFA and the susceptibility of PUFA to oxidation depends on the availability of bis-allylic hydrogen, the oxidative stability of each PUFA is inversely proportional to the number of bisallylic positions in the molecule or the degree of unsaturation of the PUFA (Miyashita, 2002). Moreover, C–H bond dissociation energies are lower at bis-allylic methylene (R_1–CH=CH–**CH_2**–CH=CH–R_2) positions in PUFA than mono-allylic (R_1–CH=CH–**CH_2**–R_2) positions in MUFA or at alkyl C–H bonds in SFA (Koppenol, 1990; Wagner et al., 1994), making bisallylic sites more thermodynamically favourable for attack. In fact, it was calculated that the propagation rate constants of FAs during lipid oxidation have an exponential correlation with the number of bis-allylic positions present (Zielinski & Pratt, 2017). Thus, lipid oxidation rates are strongly correlated to the degree of unsaturation in the FAs.

The higher concentrations of C20:5n-3 and C22:6n-3 in PL^C (1.02% and 0.83% respectively) as compared to TG^C (0.07% v.s. 0.06% respectively) could have compensated for the lower amount of C18:3n-3 (1.69% v.s. 3.07%) in terms of oxidative reactivity. Thus, it was not

surprising that n-3 derived lipid oxidation products such as 1-pentanol and E-2-(2-pentenyl) furan were present in significantly higher quantities in CPL samples, as these have been reported to be the lipid oxidation products of C20:5n-3 and C22:6n-3 (Kakuta et al., 2013), in addition to C18:3n-3 (Ho et al., 1978). Meanwhile, the higher concentrations of C20:4n-6 in PL^C as compared to TG^C (7.65% v.s. 0.47%) could have compensated for the lower amount of C18:2n-6 (21.8% v.s. 23.0%) and enhanced the reactivity of PL^C. Thus, it was not surprising that n-6 derived lipid oxidation products such as heptanal, 1-octen-3-one, (E)-2-nonenal, (E)-2-octenal were present in higher abundance in CPL samples, as these have been reported to be the lipid oxidation products of C20:4n-6 (Blank, 2000; Taylor & Mottram, 1990), in addition to C18:2n-6 (Cossignani et al., 2014).

On the other hand, the lack of significance in hexanal and (E,E)-2,4-decadienal between CTG and C(TG)PL samples could be due to the relative instability of these compounds. The thermal degradation of (E,E)-2,4-decadienal involves cleavage of the C=C bonds at the C^2 and C^4 positions to yield (E)-2-octenal and hexanal respectively (Nawar, 1984; Zamora et al., 2015). Hexanal could in turn undergo extensive breakdown and participate in reactions resulting in the formation of esters and lactones, some of which might be formed from fragment recombination (Palamand & Dieckmann, 1974). Although not quantified in this study, both of these compounds could also be oxidised to their corresponding acids (i.e. hexanoic acid and decanoic acids) (Michalski & Hammond, 1972).

The n-9 family of FAs mainly gave rise to saturated aldehydes and alcohols. Although there was a significantly lower concentration of n-9 FA in PL^C as compared to TG^C, there were more n-9 derived compounds, namely octanal, nonanal, decanal, 1-octanol, 1-nonanol, found in the samples containing CPL as compared to CTG. Since all the n-9 FAs are MUFAs, the difference in reactivity of the FAs would not be related to the degree of unsaturation of the FAs and was

more likely due to the polar head groups of the PL or the position of the FAs in the lipids. It was reported that TGs with DHA in the *sn*-2 position demonstrated higher oxidative stability than those with the FA in the *sn*-1(3) (Wijesundera et al., 2008) and was attributed to the variation in physical orientation of the FA in different positions and the interaction with the hydroperoxides.

As the lipids exist in an oil-in-water emulsion, the characteristics of the interfacial area are of paramount importance as it affects the interactions between the lipids and the chemical species in the aqueous phase, such as diffusing oxygen, hydrophilic reactants, prooxidants and antioxidants (Berton-Carabin et al., 2014). It was proposed that the lack of miscibility of TG with the other components in the system could have a limiting effect on its rate of participation in reactions (Farmer & Mottram, 1990). On the other hand, PL makes an excellent emulsifier with its amphiphilic properties, owing to the presence of a hydrophilic polar phosphate head group and lipophilic hydrocarbon chains attached to a glycerol backbone. This molecular characteristic is responsible for the surfactant properties of PL, meaning the ability to decrease interfacial tension between two immiscible phases, thereby contributing to its emulsifying capacity and water dispersibility (Cui & Decker, 2016). This would result in the formation of a high interfacial area and favour the accessibility of the lipid phase to hydrophilic prooxidants in the aqueous phase (Berton-Carabin et al., 2014), which in turn promotes lipid oxidation. In addition, the adsorption of PL at the interfacial layer could produce an anionic surface charge due to the negative charges present on the phosphate head group, leading to the electrostatic attraction of metal cations (e.g. Fe^{2+}) and accelerating lipid oxidation arising from the close proximity between the FAs and prooxidants (Mcclements & Decker, 2000). Although the flasks were swirled regularly throughout the extraction to ensure homogenous heating, as in a kitchen setting, it could be observed that bubble formation was more vigorous

in the systems containing PL^C and this could also have promoted the advancement of reactions and contributed towards a higher rate of aroma generation.

3.3.4.3.2 Maillard reaction products

It is known that the Maillard reaction and lipid oxidation pathways can influence each other through enhancement or suppression effects since common intermediates exist in both reaction cascades. Maillard-lipid interactions have been studied and reviewed (Mottram, 1998; Whitfield & Mottram, 1992; Zamora & Hidalgo, 2011). In general, a decrease in the formation of S- and/or N-containing compounds was observed upon the addition of a lipid to model systems (Farmer et al., 1989; Whitfield et al., 1988) and beef (Mottram & Edwards, 1983), although a lack of significance was reported in chicken (Chen et al., 2019). However, the effect of lipid class has been less extensively investigated and the extent of interaction is dependent on a number of factors such as the reaction conditions, quantity and type of lipid present, as well as the class of volatile compounds. Farmer and Mottram (1990) studied the effect of TG and different PL classes in cysteine-ribose model systems and concluded that different lipid classes participated in different ways in the Maillard reaction and exerted different effects depending on the class of volatile compounds.

Significant differences in the abundance of some S- and N-containing compounds were found between CTG and C(TG)PL samples. As seen in Figure 3-5, there was approximately twice the amount of 5-ethyl-2,4-dimethyl-3-thiazoline formed when TG^C was replaced with PL^C, with the quantity in the CTGPL sample falling in between. 3-Thiazolines were reported to possess nutty, roasted, meaty, onion and vegetable-like odours, depending on the substituents (Elmore et al., 1997; Mussinan et al., 1976) and could be formed from the reaction of α-hydroxyketones or α-dicarbonyls with hydrogen sulfide and ammonia in the presence of aliphatic aldehydes (Elmore & Mottram, 1997). In particular, 5-ethyl-2,4-dimethyl-3-thiazoline has a savoury,

grilled meat, fatty and juicy character as reported in Chapter 2 and could be formed from 3-mercapto-2-pentanone and acetaldehyde. 3-Mercapto-2-petanone is a Maillard reaction product which could be formed from the reaction between 4-hydroxy-5-methyl-3(2H)-furanone and cysteine via the α-diketone pathway (Cerny & Davidek, 2003), or between 1,4-dideoxyosone of ribose (5-hydroxy-2,3-pentanedione) and hydrogen sulfide (originating from cysteine) (Cerny & Davidek, 2003), or thiamin degradation (Guentert et al., 1990). Meanwhile, acetaldehyde could be generated via the Strecker degradation of amino acids such as cysteine and alanine (Rizzi, 2008; Yaylayan, 2006), as well as the degradation of reactive 2-alkenals and 2,4-alkadienals such as 2-octenal and 2,4-decadienal (Zamora et al., 2015). Since the acetaldehyde precursor could be influenced by lipid oxidation, it was likely that the higher rate of lipid oxidation in the presence of PL^C would promote the formation of 5-ethyl-2,4-dimethyl-3-thiazoline and thus, result in a significantly higher abundance of this compound in both forms of isomer found in the CPL samples. Such a lack of reactive precursors in TG as compared to PL for the interaction and absorption of reactive free molecules, and consequently a reduction in the formation of long chain heterocycles was also observed by Farmer and Mottram (1990) in model systems.

In comparison to thiazoles, a closely related class of compounds, the addition of lecithin had no marked changes in its qualitative composition (Whitfield et al., 1988) and neither did the class of lipid have a significant effect on its formation (Farmer & Mottram, 1990). However, thiazoles are the oxidation products of thiazolines and a direct comparison may not be the most appropriate. On the other hand, thiazolines are less widely reported due to their susceptibility to oxidation and the harsher reaction conditions used in the studies on model systems (≥140 °C for at least an hour) as compared to those used in this work to mimic the preparation of stocks or casseroles in a kitchen setting could explain the absence of thiazolines in the former systems.

Another observation from Figure 3-5 is the presence of about twice the abundance of tetramethylpyrazine in CPL samples as compared to CTG samples. This was in contrast to 2-ethyl-3,5-dimethylpyrazine as no significant differences in quantities were found between the samples reconstituted with CTG and C(TG)PL samples Table 3-8. This latter observation corroborated with findings in model systems of cysteine and ribose, whereby a general lack of an effect of PL addition was attributed to the formation of aminoketones, which are the precursors of pyrazine formation, as minor products from the Strecker degradation of amino acids in an acidic medium (pH 5.6) (Farmer & Mottram, 1990; Whitfield et al., 1988). On the other hand, Mottram and Edwards (1983) observed a reduction of methylpyrazine and dimethylpyrazine in reconstituted lean beef extracts in the presence of PLs but not TGs. However, tetramethylpyrazine was not reported in their study and thus, the extent of any inhibition exerted by the lipids or its degradation products on these compounds is unknown. In addition, the yield of pyrazines is also dependent on the type of amino acids present, with lysine being one of the most efficient (Hwang et al., 1995). Given that lysine is one of the most abundant amino acids in the DF meat matrix (Table 3-6), this could also have an impact on how the lipids influence pyrazine formation in the system.

3.3.4.3.3 Principal component analysis (PCA)

The data on odour-active volatiles is visually represented using PCA plot as shown in Figure 3-6. Principal components 1 (PC1) and 2 (PC2) accounted for 63.8% and 20.2% of the total variation within the data respectively. The first axis separated the samples containing any of the chicken lipids while the second axis separated the samples containing PL^C, be it naturally present or reconstituted. The samples with reconstituted chicken lipids and the other matrices were displayed as opposites with the former group more closely associated with a wider range of volatiles, which is the combined result of lipid addition to a more processed matrix. In

addition, the C(TG)PL samples were more closely associated with a balanced mix of Maillard reaction and lipid oxidation products while the CTG samples were mainly associated with lipid-derived products.

3.3.5 Kinetics experiment

3.3.5.1 Effect of heating duration on volatile generation and accumulation

As observed in Table 3-9, the abundance of volatiles in the headspace of all the samples generally increased as a function of heating duration. However, the volatiles displayed different trends depending on the lipid composition and the most notable being that of (E,E)-2,4-decadienal between CTG and C(TG)PL samples (Figure 3-7). This lipid-derived unsaturated aldehyde is known to be a character impact compound which imparts fatty notes to the aroma of BC (Gasser & Grosch, 1990) and thus, an understanding of the effect of TG^C and PL^C on its net presence (formation and degradation combined) would be relevant. In CTG samples, the amount of (E,E)-2,4-decadienal increased incrementally with increasing heating duration from 14.4 ng after 30 min to 30.9 ng after 150 min. However, the opposite trend was observed for C(TG)PL samples. In CPL samples, (E,E)-2,4-decadienal was generated in significant amounts after 30 min at 567 ng but its abundance decreased exponentially upon further heating to reach 73.1 ng after 150 min. A similar trend of increase followed by decrease in quantity of (E,E)-2,4-decadienal was also observed in CTGPL samples.

2,4-Alkadienals and 2-alkenals are highly reactive and unstable compounds (Zamora et al., 2015), which are susceptible to further degradation via autooxidation, photooxidative mechanism and thermal decomposition in which they form shorter aldehydes (Josephson & Lindsay, 1987). The degradation of (E,E)-2,4-decadienal produces hexanal and (E)-2-octenal (Nawar, 1984; Zamora et al., 2015), which were observed to increase in abundance with

increasing heating duration. In CPL samples, 278 ng of (E,E)-2,4-decadienal was lost from 30 to 60 min, whereas the concentrations of hexanal and (E)-2-octenal increased by 394 ng and 15.5 ng respectively over this period. The substantial increase (approx. 65%) in hexanal concentration and the smaller increase (approx. 40%) in (E)-2-octenal were within reason as it was reported that hexanal was usually formed to a higher extent than (E)-2-octenal during the degradation of (E,E)-2,4-decadienal (Zamora et al., 2015). Thus, it was likely that thermal decomposition of the highly reactive (E,E)-2,4-decadienal had occurred during prolonged heating. On the other hand, (E,Z)-2,4-nonadienal was observed to be formed in significantly lower abundance initially but its quantity increased gradually over the heating duration of 30 to 150 min from 10.5 to 135 ng and 3.68 to 63.8 ng in CPL and CTGPL samples respectively as observed in Figure 3-7. This might suggest that the thermal stability of (E,Z)-2,4-nonadienal was higher and it was less reactive once formed as compared to (E,E)-2,4-decadienal.

3.3.5.2 Effect of lipid class on the rate of volatile generation and accumulation

Naturally, the increase in abundance of volatiles does not continue indefinitely as precursors deplete and chemical reactions between lipid-derived volatiles and other intrinsic compounds within the meat matrix take place to form new secondary volatile and non-volatile compounds. As a result, the rate of volatile accumulation slows down leading to a decrease or stabilisation in volatile quantity with increasing heating duration. Statistical differences in the rate of volatile accumulation between the different samples were observed with some examples illustrated in Figure 3-7**.** The rate of volatile accumulation in C(TG)PL samples was significantly higher as compared to CTG samples, with the former group reaching equilibrium at a faster rate than the latter and was particularly evident after 60 min of heating. Even after prolonged heating (120 or 150 min), the quantity of volatiles present in CTG samples was only a fraction of the highest amount achieved in C(TG)PL samples. This was evident for the majority of the long-chain saturated aldehydes and alkadienals.

(E,Z)-2,4-Nonadienal was only detected after 90 min of heating in the CTG samples and its highest amount was only achieved after 150 min at 7.96 ng, whereas its concentration reached 135 ng and 63.8 ng in CPL and CTGPL samples respectively after the same heating duration. Furthermore, the primary markers of n-6 FA oxidation, (E,E)-2,4-decadienal and hexanal were approximately 4 and 40 times higher respectively in CPL samples after 30 min of heating as compared to CTG samples. This clearly indicates that the rate of lipid oxidation of PL^C was significantly faster as compared to TG^C and could be attributed to the significantly higher n-6 PUFAs content (36.3% v.s. 27.4%) present in PL^C than in TG^C, especially the larger proportion of C20:4n-6 (7.65% v.s. 0.47%), which would lead to increased oxidative susceptibility and higher reactivity as discussed in Section 3.3.4.3.1.

Interestingly, there was little to no statistical differences in the rate of accumulation and quantity of the majority of the volatiles studied between CPL and CTGPL samples despite the latter only containing half the amount of PL^C as the former. In some cases, volatile abundance in CTGPL samples even exceeded that in CPL samples, which was observed for the n-6 PUFA derived volatiles, (E)-2-octenal and (E)-2-nonenal. Since PL^C contained a higher proportion of n-6 PUFAs as compared to TG^C, the CPL samples would contain more n-6 precursors for the generation of (E)-2-octenal and (E)-2-nonenal than CTGPL samples. However, within 60 min of heating, the concentration of (E)-2-octenal and (E)-2-nonenal in CTGPL exceeded that in CPL samples (71.8 ng v.s. 54.3 ng and 34.4 ng v.s. 17.7 ng respectively). In CPL samples, maximum amounts of (E)-2-octenal and (E)-2-nonenal were achieved within 60 and 30 min respectively. Likewise, the maximum quantities of both volatiles in CTGPL were achieved and maintained at equilibrium within 60 min. This demonstrated that lipid oxidation rate of n-6 PUFAs in TG^C was boosted in the presence of PL^C, allowing the maximum generation of

volatiles from both lipids in CTGPL samples within 60 min under the present experimental conditions.

As discussed in Section 3.3.4.3.1, this could be attributed to the amphiphilic nature of PLs, which not only increased the diffusion rate of oxygen but also the dispersion of TGs within the aqueous matrix. By the action of PL, the lipid oxidation substrates were brought into close contact with each other and thus, lipid oxidation rate was increased. Moreover, once the initial stage of lipid oxidation had taken place, leading to the formation of alkyl radicals from highly unsaturated PUFAs such as those in PLs, the accumulation of alkyl radicals could promote the oxidation and degradation of the less reactive FAs such as the MUFAs and SFAs in TGs, thereby accelerating the subsequent propagation steps in the lipid oxidation chain reaction (Elmore et al., 1999). Such prooxidant effect of PLs was also previously reported in different food systems. For example, it was reported that the addition of as little as 0.3 g kg^{-1} of PLs in purified soybean oil could increase oil oxidation rates during storage (Yoon and Min, 1987).

3.4 Conclusion

Using a defatted matrix with the controlled addition of lipids to obtain a system as close to authentic meat as possible, coupled with an aroma extraction method which mimicked cooking in a kitchen setting, this work had elucidated the role of TG and PL in the thermal generation of key volatiles in BC aroma. Due to differences in FA composition and chemical properties, lipid class was found to have impact on the extent of the lipid oxidation, as well as exerted some influence on the Maillard reaction as observed in the higher quantity of S- and/or N-containing compounds produced in systems containing C(TG)PL as compared to CTG in the LN-SDE experiment. Besides demonstrating the higher efficiency of PL^C in generating lipid-derived volatiles, the kinetics experiment had also established the catalytic role of PLs in the lipid oxidation of the less reactive TGs. The knowledge that small quantities of PLs could be

used to catalyse the oxidation of TGs at an adequate rate to maximise the thermal generation of key volatiles from TGs would be a useful cost-saving strategy for manufacturers.

3.5 References

Aksoy, A., Karasu, S., Akcicek, A., & Kayacan, S. (2019). Effects of different drying methods on drying kinetics, microstructure, color, and the rehydration ratio of minced meat. *Foods, 8*(6), 216.

Aliani, M., & Farmer, L. J. (2002). Postcolumn derivatization method for determination of reducing and phosphorylated sugars in chicken by high performance liquid chromatography. *Journal of Agricultural and Food Chemistry, 50*(10), 2760-2766.

Aliani, M., & Farmer, L. J. (2005). Precursors of chicken flavor. I. Determination of some flavor precursors in chicken muscle. *Journal of Agricultural and Food Chemistry, 53*(15), 6067-6072.

Bannon, C. D., Breen, G. J., Craske, J. D., Hai, N. T., Harper, N. L., & O'Rourke, K. L. (1982). Analysis of fatty acid methyl esters with high accuracy and reliability: III. Literature review of and investigations into the development of rapid procedures for the methoxide-catalysed methanolysis of fats and oils. *Journal of Chromatography A, 247*(1), 71-89.

Berton-Carabin, C. C., Ropers, M.-H., & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. *Comprehensive reviews in food science and food safety, 13*(5), 945-977.

Blair, R., Shannon, D. W. F., McNab, J. M., & Lee, D. J. W. (1972). Effects on chick growth of adding glycine, proline, glutamic acid or diammonium citrate to diets containing crystalline essential amino acids. *British Poultry Science, 13*(3), 215-228.

Blank, I., Lin, J., & Fay, L.B. (2000). Aroma impact compounds formed by autoxidation of arachidonic acid. In P. Schieberle, and Engel, K. H. (Ed.), *Frontiers of Flavour Science* (pp. 3-9). Garching: Deutsche Forschungsanstalt für Lebensmittelchemie.

Brash, A. R. (2001). Arachidonic acid as a bioactive molecule. *The Journal of clinical investigation, 107*(11), 1339-1345.

Cerny, C., & Davidek, T. (2003). Formation of aroma compounds from ribose and cysteine during the maillard reaction. *Journal of Agricultural and Food Chemistry, 51*(9), 2714-2721.

Chaintreau, A. (2001). Simultaneous distillation–extraction: from birth to maturity—review. *Flavour and Fragrance Journal, 16*(2), 136-148.

Chen, D.-W., Balagiannis, D. P., & Parker, J. K. (2019). Use of egg yolk phospholipids to generate chicken meat odorants. *Food Chemistry, 286*, 71-77.

Cortinas, L., Villaverde, C., Galobart, J., Baucells, M. D., Codony, R., & Barroeta, A. C. (2004). Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. *Poultry Science, 83*(7), 1155-1164.

Cossignani, L., Giua, L., Simonetti, M. S., & Blasi, F. (2014). Volatile compounds as indicators of conjugated and unconjugated linoleic acid thermal oxidation. *European Journal of Lipid Science and Technology, 116*(4), 407-412.

Cui, L., & Decker, E. A. (2016). Phospholipids in foods: prooxidants or antioxidants? *Journal of the Science of Food and Agriculture, 96*(1), 18-31.

Dashdorj, D., Amna, T., & Hwang, I. (2015). Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: an overview. *European Food Research and Technology, 241*(2), 157-171.

De Carvalho, C. C. C. R., & Caramujo, M. J. (2018). The Various Roles of Fatty Acids. *Molecules, 23*(10), 2583.

Dominguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants (Basel), 8*(10), 429.

Elmore, J. S., & Mottram, D. S. (1997). Investigation of the reaction between ammonium sulfide, aldehydes, and α-hydroxyketones or α-dicarbonyls to form some lipid-Maillard interaction products found in cooked beef. *Journal of Agricultural and Food Chemistry, 45*(9), 3595-3602.

Elmore, J. S., Mottram, D. S., Enser, M., & Wood, J. D. (1997). Novel thiazoles and 3-thiazolines in cooked beef aroma. *Journal of Agricultural and Food Chemistry, 45*(9), 3603-3607.

Elmore, J. S., Mottram, D. S., Enser, M., & Wood, J. D. (1999). Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *Journal of Agricultural and Food Chemistry, 47*(4), 1619-1625.

Erickson, M. C. (2002). Lipid oxidation of muscle foods. In C. C. Akoh & D. B. Min (Eds.), *Food Lipids: Chemistry, Nutrition, and Biotechnology* (3rd ed., pp. 321-364). Boca Raton: CRC Press.

Fan, M., Xiao, Q., Xie, J., et al. (2018). Aroma compounds in chicken broths of Beijing Youji and commercial broilers. *Journal of Agricultural and Food Chemistry, 66*(39), 10242-10251.

Farkaš, P., Sádecká, J., Kováč, M., Siegmund, B., Leitner, E., & Pfannhauser, W. (1997). Key odourants of pressure-cooked hen meat. *Food Chemistry, 60*(4), 617-621.

Farmer, L. J., & Mottram, D. S. (1990). Interaction of lipid in the maillard reaction between cysteine and ribose: The effect of a triglyceride and three phospholipids on the volatile products. *Journal of the Science of Food and Agriculture, 53*(4), 505-525.

Farmer, L. J., Mottram, D. S., & Whitfield, F. B. (1989). Volatile compounds produced in maillard reactions involving cysteine, ribose and phospholipid. *Journal of the Science of Food and Agriculture, 49*(3), 347-368.

Ferioli, F., & Caboni, M. F. (2010). Composition of phospholipid fraction in raw chicken meat and pre-cooked chicken patties: influence of feeding fat sources and processing technology. *European Food Research and Technology, 231*(1), 117-126.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry, 226*(1), 497-509.

Friel, E. N., Linforth, R. S. T., & Taylor, A. J. (2000). An empirical model to predict the headspace concentration of volatile compounds above solutions containing sucrose. *Food Chemistry, 71*(3), 309-317.

Gasser, U., & Grosch, W. (1990). Primary odorants of chicken broth. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 190*(1), 3-8.

Givens, D. I., Gibbs, R. A., Rymer, C., & Brown, R. H. (2011). Effect of intensive vs. free range production on the fat and fatty acid composition of whole birds and edible portions of retail chickens in the UK. *Food Chemistry, 127*(4), 1549-1554.

Graber, G., Allen, N. K., & Scott, H. M. (1970). Proline essentiality and weight gain. *Poultry Science, 49*(3), 692-697.

Guentert, M., Bruening, J., Emberger, R., et al. (1990). Identification and formation of some selected sulfur-containing flavor compounds in various meat model systems. *Journal of Agricultural and Food Chemistry, 38*(11), 2027-2041.

He, W., Li, P., & Wu, G. (2021). Amino acid nutrition and metabolism in chickens. In G. Wu (Ed.), *Amino acids in nutrition and health: Amino acids in the nutrition of companion, zoo and farm animals* (pp. 109-131). Cham: Springer International Publishing.

Ho, C. T., Smagula, M. S., & Chang, S. S. (1978). The synthesis of 2-(1-Pentenyl) furan and its relationship to the reversion flavor of soybean oil. *Journal of the American Oil Chemists' Society, 55*(2), 233-237.

Hwang, H. I., Hartman, T. G., & Ho, C. T. (1995). Relative reactivities of amino acids in pyrazine formation. *Journal of Agricultural and Food Chemistry, 43*(1), 179-184.

Igene, J. O., & Pearson, A. M. (1979). Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *Journal of Food Science, 44*(5), 1285-1290.

Igene, J. O., Pearson, A. M., Dugan, L. R., & Price, J. F. (1980). Role of triglycerides and phospholipids on development of rancidity in model meat systems during frozen storage. *Food Chemistry, 5*(4), 263-276.

Jahan, K., & Paterson, A. (2007). Lipid composition of retailed organic, free‐range and conventional chicken breasts. *International Journal of Food Science & Technology, 42*(3), 251-262.

Josephson, D. B., & Lindsay, R. C. (1987). Retro-aldol related degradations of 2,4-decadienal in the development of staling flavors in fried foods. *Journal of Food Science, 52*(5), 1186-1190.

Kakuta, S., Bando, Y., Nishiumi, S., Yoshida, M., Fukusaki, E., & Bamba, T. (2013). Metabolic profiling of oxidized lipid-derived volatiles in blood by gas chromatography/mass spectrometry with in-tube extraction. *Mass spectrometry (Tokyo, Japan), 2*(1), A0018-A0018.

Kerscher, R., & Grosch, W. (1998). Quantification of 2-methyl-3-furanthiol, 2-furfurylthiol, 3-mercapto-2-pentanone, and 2-mercapto-3-pentanone in heated meat. *Journal of Agricultural and Food Chemistry, 46*(5), 1954-1958.

Khan, M. I., Jo, C., & Tariq, M. R. (2015). Meat flavor precursors and factors influencing flavor precursors—A systematic review. *Meat Science, 110*(Supplement C), 278-284.

Koppenol, W. H. (1990). Oxyradical reactions: from bond-dissociation energies to reduction potentials. *FEBS Letters, 264*(2), 165-167.

Koutsidis, G. (2004). *Studies on Water-Soluble Flavour Precursors In Meat.* (Doctoral Thesis), University of Reading, Reading.

Koutsidis, G., Elmore, J. S., Oruna-Concha, M. J., Campo, M. M., Wood, J. D., & Mottram, D. S. (2008). Water-soluble precursors of beef flavour: I. Effect of diet and breed. *Meat Science, 79*(1), 124-130.

Lawrie, R. A., & Ledward, D. A. (2006). Chemical and biochemical constitution of muscle. In R. A. Lawrie & D. A. Ledward (Eds.), *Lawrie's Meat Science* (7th ed., pp. 75-127). Cambrdge: Woodhead Publishing.

Lehninger, A. L., Nelson, D. L., & Cox, M. M. (2012). Lipids. In *Principles of Biochemistry* (6th ed., pp. 343-368). New York: Macmillan Learning.

Macy, R. L., Naumann, H. D., & Bailey, M. E. (1964). Water-soluble flavor and odor precursors of meat. I. Qualitative study of certain amino acids, carbohydrates, non-amino acid nitrogen compounds, and phosphoric acid esters of beef, pork, and lamb. *Journal of Food Science, 29*(2), 136-141.

Marion, J. E., & Woodroof, J. G. (1965). Lipid fractions of chicken broiler tissues and their fatty acid composition. *Journal of Food Science, 30*(1), 38-43.

Mcclements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science, 65*(8), 1270-1282.

Michalski, S. T., & Hammond, E. G. (1972). Use of labeled compounds to study the mechanism of flavor formation in oxidizing fats. *Journal of the American Oil Chemists Society, 49*(10), 563-566.

Miyashita, K. (2002). Polyunsaturated lipid oxidation in aqueous system. In C. C. Akoh & D. B. Min (Eds.), *Food Lipids: Chemistry, Nutrition, and Biotechnology* (3rd ed., pp. 365-386). Boca Raton: CRC Press.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415-424.

Mottram, D. S., & Edwards, R. A. (1983). The role of triglycerides and phospholipids in the aroma of cooked beef. *Journal of the Science of Food and Agriculture, 34*(5), 517-522.

Mussinan, C. J., Wilson, R. A., Katz, I. R. A., Hruza, A., & Vock, M. H. (1976). Identification and flavor properties of some 3-oxazolines and 3-thiazolines isolated from cooked beef. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors* (Vol. 26, pp. 133-145): American Chemical Society.

Nawar, W. W. (1984). Chemical changes in lipids produced by thermal processing. *Journal of Chemical Education, 61*(4), 299.

Nishimura, K., Murakoshi, M., Katayama, S., & Saeki, H. (2010). Changes in solubility and thermal stability of chicken myofibrillar protein by glycosylation. *Food Science and Technology Research, 17*(1), 69-75.

Palamand, S. R., & Dieckmann, R. H. (1974). Autoxidation of n-hexanal. Identification and flavor properties of some products of autoxidation. *Journal of Agricultural and Food Chemistry, 22*(3), 503-506.

Parker, J. K. (2017). Meat. In A. Buettner (Ed.), *Springer Handbook of Odor* (pp. 191-222). Cham, Switzerland: Springer.

Pati, S., Nie, B., Arnold, R. D., & Cummings, B. S. (2016). Extraction, chromatographic and mass spectrometric methods for lipid analysis. *Biomedical Chromatography, 30*(5), 695-709.

Petracci, M., Bianchi, M., & Cavani, C. (2010). Pre-slaughter handling and slaughtering factors influencing poultry product quality. *World's Poultry Science Journal, 66*(1), 17-26.

Raes, K., & De Smet, S. (2009). Fatty Acids. In L. M. L. Nollet & F. Toldra (Eds.), *Handbook of Muscle Foods Analysis* (pp. 141-154). Boca Raton: CRC Press.

Rizzi, G. P. (2008). The strecker degradation of amino acids: Newer avenues for flavor formation. *Food Reviews International, 24*(4), 416-435.

Sahasrabudhe, M. R., Delorme, N. F., Wood, D. F., & Randall, C. J. (1985). Neutral and polar lipids in chicken parts and their fatty acid composition. *Poultry Science, 64*(5), 910-916.

Saini, R. K., Prasad, P., Shang, X., & Keum, Y. S. (2021). Advances in Lipid Extraction Methods—A Review. *International Journal of Molecular Sciences, 22*(24), 13643.

Sams, A. R. (1999). Meat quality during processing. *Poultry Science, 78*(5), 798-803.

Taylor, A. J., & Mottram, D. S. (1990). Composition and odour of volatiles from autoxidised methyl arachidonate. *Journal of the Science of Food and Agriculture, 50*(3), 407-417.

Villaverde, C., Baucells, M. D., Cortinas, L., & Barroeta, A. C. (2006). Effects of dietary concentration and degree of polyunsaturation of dietary fat on endogenous synthesis and deposition of fatty acids in chickens. *British Poultry Science, 47*(2), 173-179.

Wagner, B. A., Buettner, G. R., & Burns, C. P. (1994). Free radical-mediated lipid peroxidation in cells: oxidizability is a function of cell lipid bis-allylic hydrogen content. *Biochemistry, 33*(15), 4449-4453.

Wang, X., Li, J., Cong, J., et al. (2017). Preslaughter transport effect on broiler meat quality and post-mortem glycolysis metabolism of muscles with different fiber types. *Journal of Agricultural and Food Chemistry, 65*(47), 10310-10316.

Whitfield, F. B., & Mottram, D. S. (1992). Volatiles from interactions of Maillard reactions and lipids. *Critical Reviews in Food Science and Nutrition, 31*(1-2), 1-58.

Whitfield, F. B., Mottram, D. S., Brock, S., Puckey, D. J., & Salter, L. J. (1988). Effect of phospholipid on the formation of volatile heterocyclic compounds in heated aqueous solutions of amino acids and ribose. *Journal of the Science of Food and Agriculture, 42*(3), 261-272.

Wijesundera, C., Ceccato, C., Watkins, P., et al. (2008). Docosahexaenoic acid is more stable to oxidation when located at the sn-2 position of triacylglycerol compared to sn-1(3). *Journal of the American Oil Chemists' Society, 85*(6), 543-548.

Wood, J. D., Enser, M., Fisher, A. V., et al. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science, 78*(4), 343-358.

Yaylayan, V. A. (2006). Precursors, formation and determination of furan in food. *Journal für Verbraucherschutz und Lebensmittelsicherheit, 1*(1), 5-9.

Zamora, R., & Hidalgo, F. J. (2011). The Maillard reaction and lipid oxidation. *Lipid Technology, 23*(3), 59-62.

Zamora, R., Navarro, J. L., Aguilar, I., & Hidalgo, F. J. (2015). Lipid-derived aldehyde degradation under thermal conditions. *Food Chemistry, 174*, 89-96.

Zielinski, Z. A. M., & Pratt, D. A. (2017). Lipid peroxidation: Kinetics, mechanisms, and products. *The Journal of Organic Chemistry, 82*(6), 2817-2825.

3.6 Tables and Figures

3.6.1 Tables

Table 3-1: Composition of reconstituted samples for preliminary and kinetics experiments

Component	Weight in reconstituted samples (g)				
	FD	DF	CTG	CTGPL	CPL
Freeze-dried meat	0.255	-	-	-	-
Defatted meat	-	0.235	0.235	0.235	0.235
MCT	-	0.020	-	-	-
TG^C	-	-	0.020	0.010	-
PL^C	-	-	-	0.010	0.020
Water	0.745	0.745	0.745	0.745	0.745

MCT= Medium chain triglycerides; **TG^C**= Chicken triglycerides; **PL^C**= Chicken phospholipids; **FD**= Freeze-dried meat sample; **DF**= Defatted meat sample containing MCT; **CTG**= Defatted meat sample containing TG^C; **CTGPL**= Defatted meat sample containing TG^C and PL^C (1:1 w/w); **CPL**= Defatted meat sample containing PL^C

Table 3-2: Quantities of sugars and amino acids added back to LSA samples

Sugars and sugar phosphates ($\mu g\ g^{-1}$ wet sample)							
Ribose	Fructose	Mannose	Glucose	R5P	F6P	M6P	G6P
17.7	27.2	18.5	455	8.56	19.7	40.4	224

LSA= Defatted meat sample containing TG^C and PL^C (1:1 w/w) with the addition of sugar and amino acids; **R5P**= D-Ribose-5-phosphate disodium salt hydrate; **F6P**= D-Fructose-6-phosphate disodium salt hydrate; **M6P**= D-Mannose-6-phosphate disodium salt hydrate; **G6P**= D-Glucose-6-phosphate disodium salt hydrate

Amino acids (mg g^{-1} sample)										
Phe	Trp	Leu	Ile	Met	Tyr	Val	Cys	Pro	Ala	
80.8	-	160	91.4	53.3	65.6	120	101	285	133	
Glu	Thr	Gly	Ser	Asn	Gln	Lys	Asp	His	Arg	Cystine
127	5.58	83.1	133	-	19.1	89.6	69.8	85.4	174	46.9

Table 3-3: Composition of reconstituted samples for LN-SDE experiment

Component	Weight in reconstituted samples (g)				
	FD	DF	CTG	CTGPL	PL
Freeze-dried meat	6.3	-	-	-	-
Defatted meat	-	5.8	5.8	5.8	5.8
MCT	-	0.5	-	-	-
TG^C	-	-	0.5	0.25	-
PL^C	-	-	-	0.25	0.5
Water	18.7	18.7	18.7	18.7	18.7

MCT= Medium chain triglycerides; **TG^C**= Chicken triglycerides; **PL^C**= Chicken phospholipids; **FD**= Freeze-dried meat sample; **DF**= Defatted meat sample containing MCT; **CTG**= Defatted meat sample containing TG^C; **CTGPL** = Defatted meat sample containing TG^C and PL^C (1:1 w/w); **CPL**= Defatted meat sample containing PL^C

Table 3-4: Fatty acid composition of lipids (% of measured fatty acids; n=3)

Fatty acid	TG^C	PL^C	SEM^1	p^2	Fatty acid	TG^C	PL^C	SEM^1	p^2

SFA					**PUFA**				
C10:0	0.03	0.10	0.02	***	C18:3n-3	3.07	1.69	0.32	**
C12:0	0.03	n.d.	0.01	***	C20:3n-3	0.05	0.28	0.05	***
C14:0	0.51	0.35	0.04	*	C20:5n-3	0.07	1.02	0.22	***
C15:0	0.09	0.13	0.01	***	C22:6n-3	0.06	0.83	0.18	***
C16:0	22.1	26.2	0.96	**	*t9,t12*-C18:2	0.03	n.d.	0.01	**
C17:0	0.13	0.19	0.01	***	*c9,c12*-C18:2	23.0	21.8	0.30	*
C18:0	5.93	11.1	1.15	***	C18:3n-6	0.19	0.10	0.03	n.s.
C20:0	0.08	0.04	0.01	n.s.	C20:2n-6	0.24	1.13	0.20	***
C21:0	0.02	0.24	0.06	*	C20:3n-6	0.22	1.42	0.27	***
C22:0	0.03	n.d.	0.01	*	C20:4n-6	0.47	7.65	1.61	***
C24:0	0.02	0.12	0.03	n.s.	C22:2n-6	n.d.	0.36	0.08	**
MUFA					**Total**				
C16:1n-7	4.33	1.22	0.69	***	SFA	29.0	38.5	2.10	*
C14:1n-9	0.13	n.d.	0.03	***	MUFA	43.6	25.0	4.13	***
t9-C18:1	0.21	0.13	0.02	***	PUFA	27.4	36.3	2.00	***
c9-C18:1	38.4	21.7	3.74	***	n-3	3.24	3.82	0.15	***
C20:1n-9	0.44	0.36	0.02	**	n-6	24.1	32.5	1.87	***
C22:1n-9	0.03	n.d.	0.01	***	n-9	39.2	23.8	3.43	***
C24:1n-9	0.01	1.60	0.36	***					

TG[C]= Chicken triglyceride; **PL**[C]= Chicken phospholipid; **FA**= Fatty acid; **SFA**= C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C24:0; **MUFA**= C14:1, C16:1n-7, t9-C18:1, c9-C18:1, C20:1n-9, C22:1n-9, C24:1n-9; **PUFA**= t9,t12-C18:2, c9,c12-C18:2, C18:3n-3, C18:3n-6, C20:2n-6, C20:3n-3, C20:3n-6, C20:4n-6, C20:5n-3, C22:2n-6, C22:6n-3; n.d.= not detected

[1] Standard error mean
[2] Probability that there is a significant difference between means as determined by Fisher's LSD test; n.s. = not significant; *, ** and *** denote significant differences at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ respectively

Table 3-5: Concentration of free sugars and sugar phosphates (mg 100 g^{-1} wet weight) in freeze-dried and defatted meat (n=3)

Compound	Freeze-dried meat	Defatted meat	SEM[1]	*p*[2]
Ribose	2.11	0.33	0.40	***
Fructose	3.74	1.02	0.67	*
Mannose	2.20	0.35	0.42	**
Glucose	52.0	6.44	10.3	***
R5P	1.02	0.16	0.20	*
F6P	3.04	1.08	0.50	*
M6P	5.20	1.16	0.92	**
G6P	24.5	2.10	5.02	***

R5P= D-Ribose-5-phosphate disodium salt hydrate; **F6P**= D-Fructose-6-phosphate disodium salt hydrate; **M6P**= D-Mannose-6-phosphate disodium salt hydrate; **G6P**= D-Glucose-6-phosphate disodium salt hydrate

[1] Standard error mean
[2] Probability that there is a significant difference between means as determined by Fisher's LSD test; *, ** and *** denote significant differences at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ respectively

Table 3-6: Concentration of amino acids (mg 100 g^{-1} wet weight) in freeze-dried and defatted meat (n=4)

Amino acid	Freeze-dried meat	Defatted meat	SEM[1]	*p*[2]
Alanine	28.6	15.3	2.54	***
Arginine	38.6	21.1	3.33	***
Asparagine	0.67	1.02	0.08	*
Aspartic acid	12.8	5.83	1.33	***
Cysteine	16.7	6.62	1.90	***
Cystine	23.9	19.2	0.94	**
Glutamic acid	33.0	20.3	2.43	***
Glutamine	5.92	4.02	0.37	***
Glycine	37.8	29.5	1.74	*
Histidine	25.0	16.5	1.65	***
Isoleucine	17.2	8.06	1.75	***
Leucine	34.0	18.0	3.07	***
Lysine	41.0	32.1	1.85	*
Methionine	12.8	7.48	1.02	***
Phenylalanine	17.9	9.82	1.54	***
Proline	87.4	58.8	5.61	**
Serine	46.8	33.5	2.66	**
Threonine	0.69	0.13	0.11	***
Tryptophan	n.d.	0.06	0.01	*
Tyrosine	22.0	15.4	1.31	**
Valine	22.9	10.9	2.29	***

n.d.= not detected

[1] Standard error mean

[2] Probability that there is a significant difference between means as determined by Fisher's LSD test; *, ** and *** denote significant differences at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ respectively

Table 3-7: Mean quantities (approx. ng) of the key volatile compounds identified by HS-SPME-GC-MS in the preliminary experiment (n=3)

Compound	LRI[1]	ID[2]	Mean quantities of volatiles (approx. ng)[3]						SEM[4]	p[5]
			FD	DF	TG	PL	TGPL	LSA		
Maillard reaction products										
3-(Methylthio)propanal	908	A	16.4^{ab}	5.19^{c}	20.1^{a}	7.82^{bc}	14.4^{abc}	12.7^{abc}	1.62	*
2-Phenylacetaldehyde	1053	A	13.8^{bc}	8.22^{c}	28.4^{a}	19.7^{ab}	29.1^{a}	26.5^{a}	2.26	**
n-6 lipid derived										
Hexanal	800	A	181^{c}	696^{c}	1610^{b}	2500^{ab}	2370^{b}	3400^{a}	285	***
1-Hexanol	872	A	90.4^{a}	7.00^{b}	20.1^{b}	13.6^{b}	17.7^{b}	21.0^{b}	9.01	*
Heptanal	902	A	51.6^{c}	46.3^{c}	132^{b}	241^{a}	231^{a}	286^{a}	23.8	***
1-Octen-3-ol	981	A	30.8^{d}	101^{d}	214^{c}	479^{b}	497^{b}	579^{a}	51.8	***
2-Pentylfuran	994	A	20.5^{c}	148^{c}	175^{c}	1120^{a}	694^{b}	838^{b}	101	***
(E)-2-Octenal	1062	A	5.43^{c}	7.26^{c}	47.5^{b}	95.4^{a}	98.9^{a}	93.7^{a}	10.0	***
(E)-2-Nonenal	1164	A	8.52^{c}	5.18^{c}	27.1^{b}	36.5^{ab}	43.6^{a}	47.0^{a}	4.29	***
(E)-2-Decenal	1265	A	n.d.^{b}	1.41^{b}	14.0^{b}	62.2^{a}	58.2^{a}	75.3^{a}	8.75	**
(E,E)-2,4-Decadienal	1322	A	n.d.^{b}	n.d.^{b}	n.d.^{b}	56.7^{a}	76.8^{a}	97.1^{a}	11.2	**
n-9 lipid derived										
Octanal	1004	A	71.9^{d}	66.1^{d}	170^{c}	438^{ab}	403^{b}	483^{a}	42.9	***
1-Octanol	1070	A	14.0^{d}	25.1^{d}	71.8^{c}	123^{b}	156^{a}	182^{a}	15.6	***
Nonanal	1106	A	250^{d}	497^{d}	895^{c}	2100^{ab}	1940^{b}	2380^{a}	204	***
Decanal	1207	A	18.5^{d}	45.8^{d}	88.3^{d}	523^{a}	321^{c}	404^{b}	47.7	***
Ketones										
2-Heptanone	892	A	4.66^{c}	12.7^{c}	27.0^{b}	34.0^{ab}	32.4^{ab}	38.6^{a}	3.11	***
2,3-Octanedione	984	A	7.21^{d}	27.9^{d}	73.9^{c}	153^{b}	179^{b}	211^{a}	18.9	***
Long chain aldehydes										
Undecanal	1308	A	7.93^{d}	14.9^{cd}	49.0^{c}	122^{a}	83.9^{b}	104^{ab}	11.1	***
Tridecanal	1512	A	7.12^{c}	21.7^{c}	25.8^{bc}	203^{a}	63.6^{bc}	84.2^{b}	17.4	***

Compound	LRI[1]	ID[2]	Mean quantities of volatiles (approx. ng)[3]						SEM[4]	p[5]
			FD	DF	TG	PL	TGPL	LSA		
Tetradecanal	1615	B	12.1[b]	81.8[b]	34.8[b]	792[a]	195[b]	247[b]	71.7	***
Pentadecanal	1720	B	10.2[b]	93.6[b]	18.9[b]	1310[a]	273[b]	297[b]	126	**
Hexadecanal	1824	B	264[b]	94.5[b]	36.5[b]	8650[a]	2540[b]	2180[b]	847	**
(Z)-13-Octadecenal	2012	B	1.64[b]	3.42[b]	n.d.[b]	644[a]	117[b]	101[b]	67.0	**
Octadecanal	2031	B	2.29[b]	3.05[b]	n.d.[b]	385[a]	80.6[b]	79.6[b]	38.9	**

FD= Freeze-dried meat sample; **DF**= Defatted meat sample containing medium chain triglycerides; **CTG**= Defatted meat sample containing chicken triglycerides; **CPL**= Defatted meat sample containing chicken phospholipids; **CTGPL**= Defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w); **LSA**= Defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w) with the addition of sugar and amino acids

[1] Linear retention indices determined on DB-5 column

[2] Confirmation of identity where A = MS and LRI agree with those of authentic compounds; B = MS agrees with reference spectrum in NIST 14 MS database and LRI agrees with literature values in the Chemistry Web Book database

[3] Means of 3 replicates where the same letters within each row indicate no significant differences (p = 0.05) as determined by Fisher's LSD test; n.d. = not detected

[4] Standard error mean

[5] Probability that there is a significant difference between means as determined by Fisher's LSD test; *, ** and *** denote significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively

Table 3-8: Mean quantities (approx. ng) of the key volatile compounds identified in the LN-SDE experiment (n=4)

Compound	LRI[1]	ID[2]	Mean quantities of volatiles (approx. ng)[3]						SEM[4]	p[5]
			FMM	FD	DF	CTG	CTGPL	CPL		
Maillard reaction products										
3-Methylbutanal†	657	A	17.6^{c}	29.3^{c}	30.0^{c}	54.3^{b}	53.8^{b}	73.8^{a}	4.56	***
2-Methylbutanal†	664	A	135^{b}	238^{a}	125^{b}	180^{ab}	219^{a}	183^{ab}	12.4	*
2,3-Pentanedione†	696	A	n.d.^{c}	154^{b}	155^{b}	426^{a}	436^{a}	484^{a}	42.1	***
Dimethyl disulfide†	746	A	795^{a}	729^{a}	623^{a}	774^{a}	1030^{a}	655^{a}	64.2	n.s.
3-(Methylthio)propanal†	912	A	206^{a}	275^{a}	88.1^{b}	252^{a}	239^{a}	272^{a}	16.6	**
Dimethyl trisulfide†	984	A	1440^{ab}	1310^{ab}	1030^{b}	1450^{ab}	2120^{a}	1430^{ab}	130	n.s.
2-Phenylacetaldehyde†	1058	A	1090^{c}	1220^{c}	1500^{bc}	2040^{ab}	2450^{a}	2660^{a}	146	***
5-Ethyl-2,4-dimethyl-3-thiazoline (I)†	1080	A	86.6^{a}	38.1^{c}	34.7^{c}	28.2^{c}	45.2^{bc}	63.6^{ab}	5.07	***
2-Ethyl-3,5-dimethylpyrazine†	1084	A	60.1^{c}	139^{bc}	163^{b}	275^{a}	342^{a}	346^{a}	24.4	***
Tetramethylpyrazine†	1091	A	322^{a}	300^{a}	78.3^{c}	70.9^{c}	99.3^{c}	144^{b}	21.8	***
5-Ethyl-2,4-dimethyl-3-thiazoline (II)†	1099	A	111^{a}	59.5^{c}	68.5^{bc}	54.3^{c}	95.7^{ab}	107^{a}	6.08	**
1,3-Benzothiazole†	1235	A	n.d.^{d}	154^{c}	24.8^{d}	113^{c}	307^{b}	480^{a}	35.6	***
n-3 lipid derived										
1-Pentanol	778	A	87.3^{b}	105^{b}	98.6^{b}	88.7^{b}	277^{a}	118^{b}	17.0	***
(E)-2-Hexenal	864	A	n.d.^{b}	n.d.^{b}	9.70^{b}	12.9^{b}	33.1^{a}	16.7^{ab}	3.21	**
(E)-2-(2-Pentenyl)furan	1001	A	n.d.^{d}	n.d.^{d}	15.1^{c}	9.90^{cd}	47.8^{a}	31.9^{b}	3.89	***
n-6 lipid derived										
Hexanal†	802	A	172^{b}	562^{b}	4790^{a}	3870^{a}	3570^{a}	4760^{a}	420	***
Heptanal†	903	A	68.7^{d}	192^{d}	514^{c}	581^{bc}	725^{ab}	870^{a}	61.7	***
(E)-2-Heptenal	963	A	70.1^{c}	3.60^{c}	243^{b}	271^{b}	410^{a}	313^{b}	30.6	***
1-Octen-3-one†	978	A	n.d.^{c}	n.d.^{c}	n.d.^{c}	34.0^{b}	40.4^{b}	50.6^{a}	4.52	***

Compound	LRI[1]	ID[2]	Mean quantities of volatiles (approx. ng)[3]						SEM[4]	p[5]
			FMM	FD	DF	CTG	CTGPL	CPL		
1-Octen-3-ol†	983	A	63.1^{c}	141^{c}	659^{b}	709^{b}	993^{a}	776^{b}	72.8	***
2-Pentylfuran	992	A	25.7^{d}	76.4^{d}	487^{bc}	425^{c}	1110^{a}	733^{b}	85.0	***
(E)-2-Octenal	1063	A	15.0^{c}	93.6^{c}	718^{b}	679^{b}	1180^{a}	837^{b}	88.0	***
(E)-2-Octen-1-ol	1071	A	47.0^{c}	29.3^{c}	66.2^{c}	63.8^{c}	296^{a}	151^{b}	20.5	***
(E)-3-Nonen-2-one	1144	A	n.d.^{d}	n.d.^{d}	6.60^{cd}	9.90^{c}	37.7^{a}	24.7^{b}	3.00	***
(E)-2-Nonenal†	1168	A	n.d.^{d}	n.d.^{d}	220^{c}	205^{c}	266^{b}	354^{a}	28.0	***
(E)-2-Decenal	1266	A	67.5^{cd}	33.0^{d}	115^{bcd}	151^{bc}	253^{a}	201^{ab}	19.6	**
(E,E)-2,4-Decadienal†	1333	A	37.8^{c}	70.9^{c}	199^{b}	379^{a}	353^{a}	399^{a}	31.4	***
n-9 lipid derived										
Octanal†	1007	A	73.8^{d}	159^{d}	394^{c}	325^{c}	550^{b}	796^{a}	52.4	***
1-Octanol	1073	A	11.9^{d}	65.2^{cd}	48.1^{cd}	82.2^{c}	599^{a}	319^{b}	44.3	***
Nonanal†	1107	A	2410^{ab}	1200^{b}	2260^{ab}	2640^{ab}	3860^{a}	3720^{a}	283	*
1-Nonanol	1173	A	n.d.^{c}	n.d.^{c}	58.6^{c}	72.1^{c}	685^{a}	370^{b}	55.6	***
Decanal†	1209	A	84.5^{d}	72.2^{d}	682^{b}	346^{c}	763^{b}	941^{a}	72.5	***
Ketones										
2-Heptanone	891	A	3.60^{d}	25.8^{d}	161^{c}	189^{bc}	360^{a}	269^{b}	28.3	***
2,3-Octanedione	988	A	2.70^{d}	69.6^{c}	142^{ab}	124^{abc}	184^{a}	105^{bc}	14.1	***
2-Octanone	992	A	n.d.^{d}	n.d.^{d}	16.0^{c}	17.2^{c}	40.6^{a}	29.5^{b}	3.28	***
2-Nonanone	1093	A	n.d.^{e}	17.9^{de}	56.0^{c}	46.2^{cd}	192^{a}	126^{b}	14.3	***
2-Decanone	1194	A	2.80^{e}	13.1^{de}	43.2^{cd}	50.8^{c}	264^{a}	149^{b}	19.6	***
2-Undecanone	1293	B	4.30^{d}	16.4^{c}	27.4^{bc}	35.8^{b}	88.1^{a}	76.2^{a}	6.55	***
Long chain aldehydes										
Tetradecanal	1618	B	185^{c}	301^{c}	975^{c}	292^{c}	4570^{a}	3300^{b}	370	***
Pentadecanal	1715	B	273^{b}	1230^{b}	543^{b}	2310^{b}	11500^{a}	9230^{a}	978	***
Hexadecanal	1818	B	20800^{bc}	79000^{b}	13900^{c}	47800^{bc}	233000^{a}	231000^{a}	20700	***

FMM= Frozen minced meat sample; **FD**= Freeze-dried meat sample; **DF**= Defatted meat sample containing medium chain triglycerides; **CTG**= Defatted meat sample containing chicken triglycerides; **CTGPL**= Defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w); **CPL**= Defatted meat sample containing chicken phospholipids

†Odour active compound
[1] Linear retention indices determined on DB-5 column
[2] Confirmation of identity where A = MS and LRI agree with those of authentic compounds; B = MS agrees with reference spectrum in NIST 14 MS database and LRI agrees with literature values in the Chemistry Web Book database
[3] Means of 4 replicates where the same letters within each row indicate no significant differences (p = 0.05) as determined by Fisher's LSD test; n.d. = not detected
[4] Standard error mean
[5] Probability that there is a significant difference between means as determined by Fisher's LSD test; n.s. = no significant difference; *, ** and *** denote significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively

Table 3-9: Mean quantities (ng or approx. ng) of the key volatile compounds identified in the kinetics experiment (n=4)

Compound	LRI[1]	ID[2]	Mean quantities of volatiles (ng or approx. ng)[3]															SEM[4]	p[5]
			CTG					CTGPL					CPL						
			30	60	90	120	150	30	60	90	120	150	30	60	90	120	150		
n-6 lipid derived																			
Hexanal	800	A	147^{f}	184^{ef}	214^{ef}	367^{def}	439^{de}	462^{de}	1280^{abc}	1260^{abc}	1370^{ab}	1350^{ab}	606^{d}	1000^{c}	1250^{abc}	1510^{a}	1200^{bc}	67.7	***
1-Hexanol	869	A	1.91^{f}	2.48^{ef}	2.94^{def}	5.06^{def}	5.68^{de}	4.59^{def}	15.0^{b}	15.9^{ab}	16.0^{ab}	16.9^{ab}	6.54^{d}	11.2^{c}	15.4^{b}	19.5^{a}	16.6^{ab}	0.847	***
Heptanal	902	A	15.3^{g}	32.0^{fg}	44.4^{efg}	86.1^{de}	103^{d}	61.8^{def}	207^{bc}	220^{ab}	219^{ab}	225^{ab}	96.7^{d}	170^{c}	220^{ab}	257^{a}	210^{bc}	11.1	***
1-Octen-3-ol	979	A	26.8^{f}	34.0^{f}	36.8^{f}	56.6^{f}	65^{ef}	124^{e}	364^{bc}	368^{bc}	353^{bc}	386^{abc}	207^{d}	321^{c}	395^{ab}	452^{a}	383^{bc}	21.1	***
2-Pentylfuran	994	A	23.5^{f}	58.0^{f}	92.9^{f}	229^{f}	288^{f}	195^{f}	1180^{e}	1670^{de}	1920^{cd}	2580^{b}	467^{f}	1220^{e}	2390^{bc}	3380^{a}	3630^{a}	163	***
(E)-2-Octenal	1061	A	6.71^{j}	8.22^{ij}	12.0^{hij}	19.9^{ghi}	28.1^{fg}	23.4^{gh}	71.8^{ab}	69.5^{ab}	62.5^{bc}	75.8^{a}	38.8^{ef}	54.3^{cd}	48.4^{de}	45.0^{de}	39.8^{ef}	3.11	***
(E)-2-Nonenal	1164	A	2.67^{f}	4.89^{ef}	7.57^{def}	11.7^{bcde}	17.6^{b}	9.22^{cdef}	34.4^{a}	32.1^{a}	28.7^{a}	35.8^{a}	13.0^{bcd}	17.7^{b}	18.8^{b}	17.5^{b}	16.1^{bc}	1.45	***
(E,Z)-2,4-Nonadienal	1197	B	$n.d.^{g}$	$n.d.^{g}$	2.45^{g}	5.00^{g}	7.96^{g}	3.68^{g}	27.7^{ef}	41.7^{e}	45.8^{de}	63.8^{d}	10.5^{fg}	35.8^{e}	83.5^{c}	115^{b}	135^{a}	5.65	***
(E,E)-2,4-Decadienal	1323	A	14.4^{f}	19.4^{ef}	25.8^{ef}	32.0^{def}	30.9^{def}	239^{b}	301^{b}	149^{c}	107^{cd}	96.7^{cde}	567^{a}	289^{b}	142^{c}	104^{cd}	73.1^{cdef}	19.8	***
n-9 lipid derived																			
Octanal	1004	A	33.7^{g}	80.5^{fg}	120^{ef}	247^{d}	288^{d}	153^{e}	492^{ab}	513^{a}	489^{ab}	489^{ab}	252^{d}	378^{c}	494^{ab}	509^{a}	434^{bc}	22.5	***
1-Octanol	1070	A	5.22^{e}	19.1^{de}	26.2^{de}	61.0^{cd}	79.8^{c}	46.4^{cde}	197^{a}	206^{a}	201^{a}	225^{a}	78.4^{c}	147^{b}	202^{a}	232^{a}	215^{a}	11.3	***
Nonanal	1106	A	161^{f}	300^{ef}	354^{e}	586^{d}	720^{cd}	555^{d}	1420^{a}	1420^{a}	1310^{ab}	1270^{ab}	882^{c}	1250^{ab}	1410^{a}	1320^{ab}	1180^{b}	59.1	***
Decanal	1208	A	22.1^{g}	35.8^{fg}	42.6^{fg}	56.7^{fg}	76.6^{f}	147^{e}	322^{a}	281^{abc}	260^{bcd}	230^{d}	230^{d}	270^{bcd}	306^{ab}	280^{abc}	249^{cd}	14.4	***
Ketones																			

Compound	LRI[1]	ID[2]	Mean quantities of volatiles (ng or approx. ng)[3]														SEM[4]	p[5]
			CTG					CTGPL					CPL					
			30	60	90	120	150	30	60	90	120	150	30	60	90	120	150	
2-Heptanone	891	A	2.76^{f}	4.55^{f}	6.08^{f}	10.8^{f}	13.8^{f}	7.58^{f}	35.7^{de}	42.3^{cd}	49.7^{bc}	58.5^{ab}	11.7^{f}	27.4^{e}	48.2^{bc}	70.4^{a}	68.5^{a}	3.20	***
2,3-Octanedione	982	A	5.47^{fg}	4.75^{g}	3.93^{g}	5.41^{g}	5.46^{g}	13.4^{bc}	19.7^{a}	14.3^{b}	11.2^{cde}	10.8^{de}	13.3^{bc}	13.2^{bcd}	10.5^{e}	11.0^{cde}	7.85^{f}	0.593	***
Long chain aldehydes																			
Undecanal	1310	A	17.6^{e}	22.7^{de}	24.7^{de}	30.1^{de}	47.3^{de}	49.0^{d}	166^{a}	154^{ab}	149^{ab}	135^{b}	90.7^{c}	134^{b}	168^{a}	159^{ab}	156^{ab}	8.06	***
Dodecanal	1411	A	18.4^{d}	27.4^{d}	30.6^{d}	36.2^{d}	67.0^{d}	80.9^{d}	346^{a}	325^{ab}	312^{ab}	279^{ab}	179^{c}	254^{bc}	343^{a}	331^{ab}	318^{ab}	18.4	***
Tridecanal	1515	A	n.d.^{e}	n.d.^{e}	10.4^{e}	13.7^{e}	28.9^{e}	54.3^{de}	315^{ab}	320^{ab}	321^{ab}	284^{ab}	158^{cd}	231^{bc}	342^{a}	350^{a}	348^{a}	20.8	***
Tetradecanal	1618	B	n.d.^{d}	4.69^{d}	7.83^{d}	8.16^{d}	25.0^{d}	66.4^{d}	521^{ab}	595^{ab}	645^{ab}	576^{ab}	242^{cd}	408^{bc}	675^{a}	730^{a}	684^{a}	43.0	***
Pentadecanal	1721	B	n.d.^{e}	3.47^{e}	7.09^{de}	n.d.^{e}	12.8^{de}	53.5^{de}	418^{bc}	519^{abc}	598^{ab}	565^{ab}	281^{cd}	440^{bc}	687^{ab}	720^{a}	543^{abc}	41.7	***
Hexadecanal	1825	B	n.d.^{d}	22.0^{d}	59.2^{d}	n.d.^{d}	55.9^{d}	137^{d}	966^{cd}	1800^{bc}	2350^{bc}	2610^{b}	967^{cd}	2060^{bc}	4400^{a}	5460^{a}	4150^{a}	256	***
(Z)-13-Octadecenal	2012	B	n.d.^{d}	n.d.^{d}	n.d.^{d}	n.d.^{d}	n.d.^{d}	n.d.^{d}	37.2^{cd}	87.8^{cd}	106^{cd}	144^{c}	51.6^{cd}	142^{c}	369^{ab}	470^{a}	298^{b}	21.2	***
Octadecanal	2032	B	n.d.^{f}	n.d.^{f}	n.d.^{f}	n.d.^{f}	n.d.^{f}	n.d.^{f}	20.5^{f}	47.7^{def}	54.3^{def}	105^{cd}	24.3^{ef}	79.9^{de}	177^{ab}	223^{a}	143^{bc}	10.2	***

CTG= Defatted meat sample containing chicken triglycerides; **CTGPL**= Defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w); **CPL**= Defatted meat sample containing chicken phospholipids

[1] Linear retention indices determined on DB-5 column

[2] Confirmation of identity where A = MS and LRI agree with those of authentic compounds; B = MS agrees with reference spectrum in NIST 14 MS database and LRI agrees with literature values in the Chemistry Web Book database

[3] Means of 4 replicates where the same letters within each row indicate no significant differences (p = 0.05); n.d. = not detected

[4] Standard error mean

[5] Probability that there is a significant difference between means as determined by Fisher's LSD test; *** denotes significant differences at $p < 0.001$

3.6.2 Figures

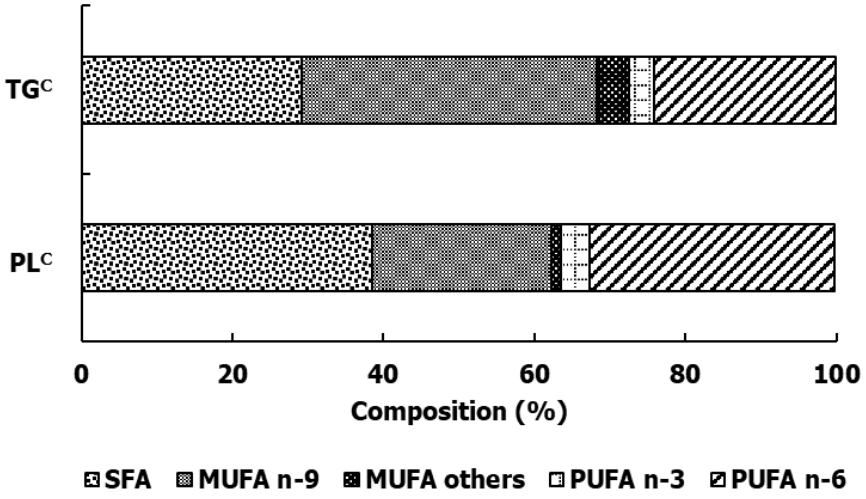


Figure 3-1: Fatty acid class composition (%) of chicken triglyceride (TG^C) and phospholipid (PL^C)

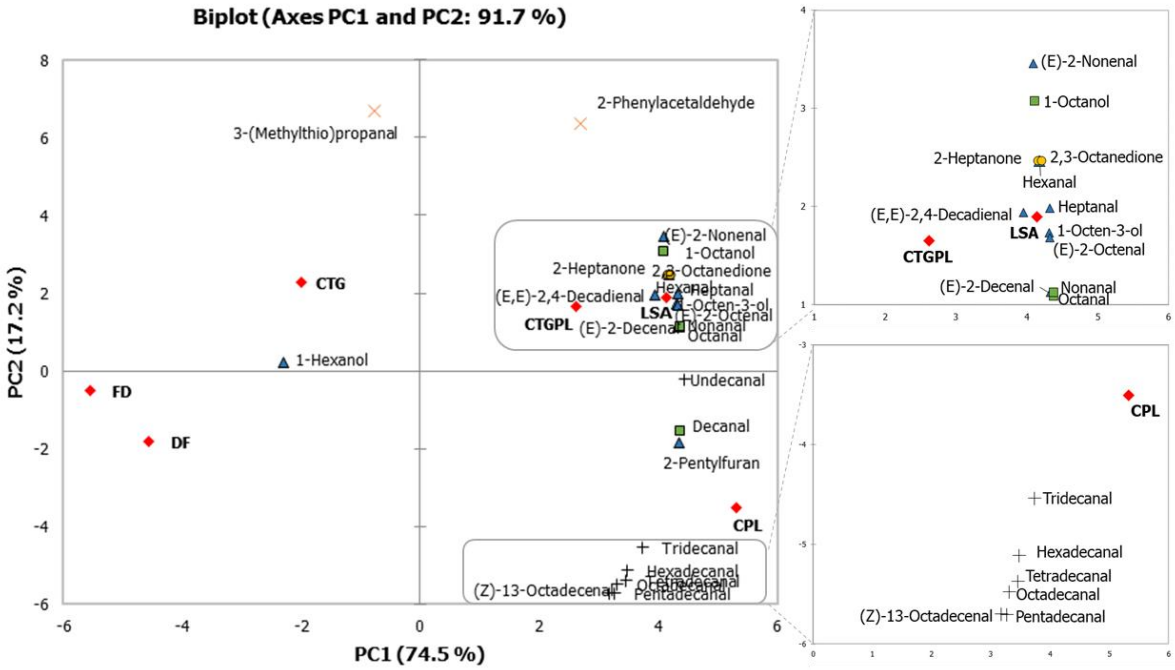


Figure 3-2: Principal component analysis of reconstituted samples (FD= Freeze-dried meat sample; DF= Defatted meat sample containing medium chain triglycerides; CTG= Defatted meat sample containing chicken triglycerides; CPL= Defatted meat sample containing chicken phospholipids; CTGPL= Defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w); LSA= Defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w) with the addition of sugars and amino acids) showing correlations with volatiles identified by SPME in preliminary experiment (X: Maillard reaction product; □: n-3 lipid degradation product; ∆: n-6 lipid degradation product; •: ketone; +: long chain aldehyde)

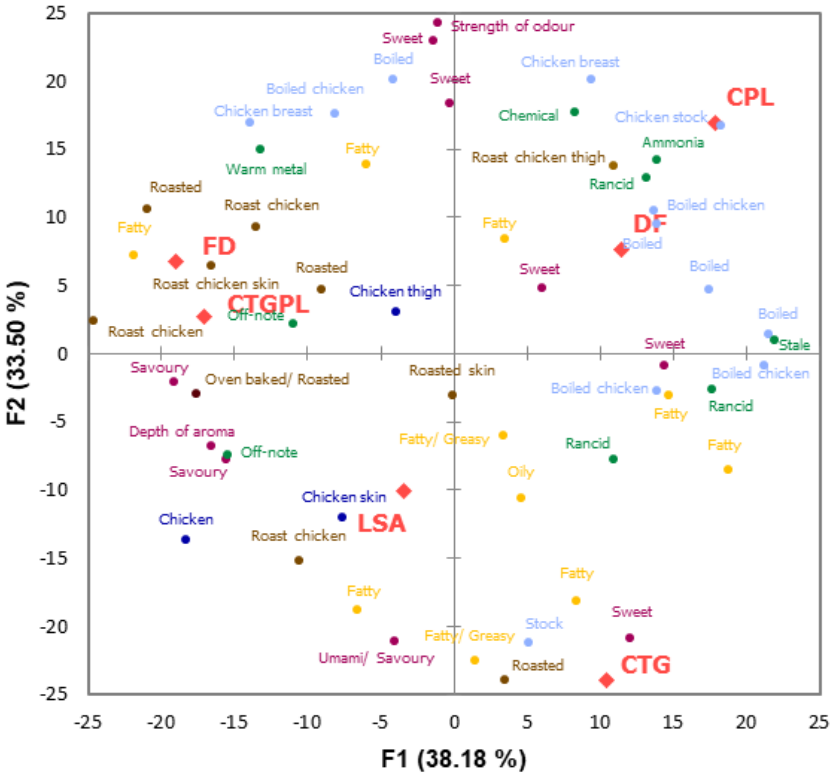


Figure 3-3: Generalised procrustes analysis of free choice profiling data from aroma evaluation of reconstituted samples (FD= Freeze-dried meat sample; DF= Defatted meat sample containing medium chain triglycerides; CTG= Defatted meat sample containing chicken triglycerides; CPL= Defatted meat sample containing chicken phospholipids; CTGPL= Defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w); LSA= Defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w) with the addition of sugars and amino acids) overlayed with odour descriptors generated by the panellists (blue: boiled/chicken; brown: roasted/baked; yellow: fatty; burgundy: pleasant (sweet/savoury); green: off-notes)

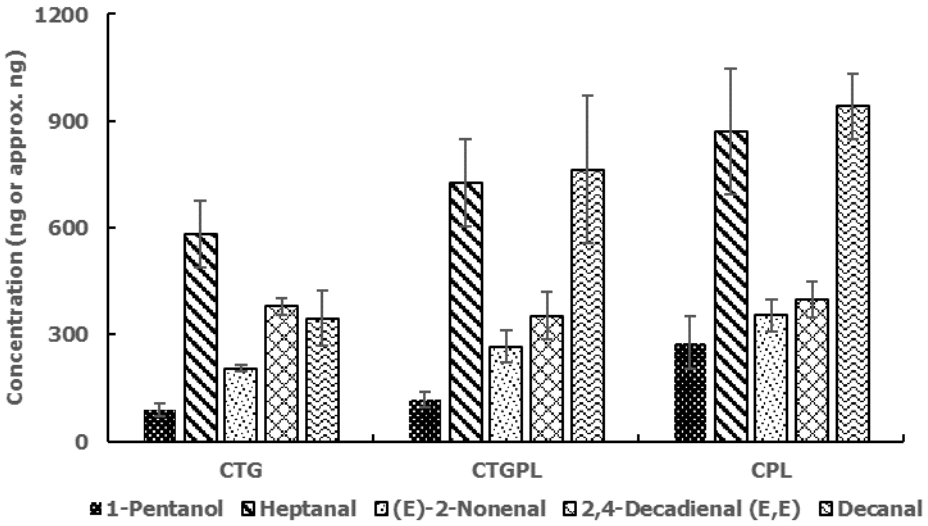


Figure 3-4: Mean quantities (approx. ng) of selected lipid-derived volatiles in LN aroma extracts of reconstituted samples (CTG= defatted meat sample containing chicken triglycerides; CTGPL= defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w); CPL= defatted meat sample containing chicken phospholipids; n=4 with error bars representing standard deviation of replicates)

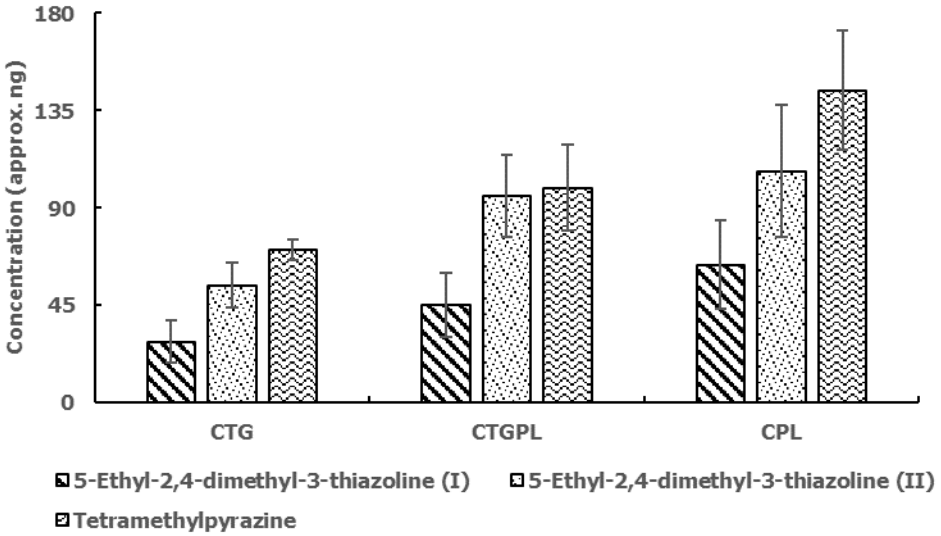


Figure 3-5: Mean quantities (approx. ng) of thiazolines and tetramethylpyrazine in LN aroma extracts of reconstituted samples (CTG= defatted meat sample containing chicken triglycerides; CTGPL= defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w); CPL= defatted meat sample containing chicken phospholipids; n=4 with error bars representing standard deviation of replicates)

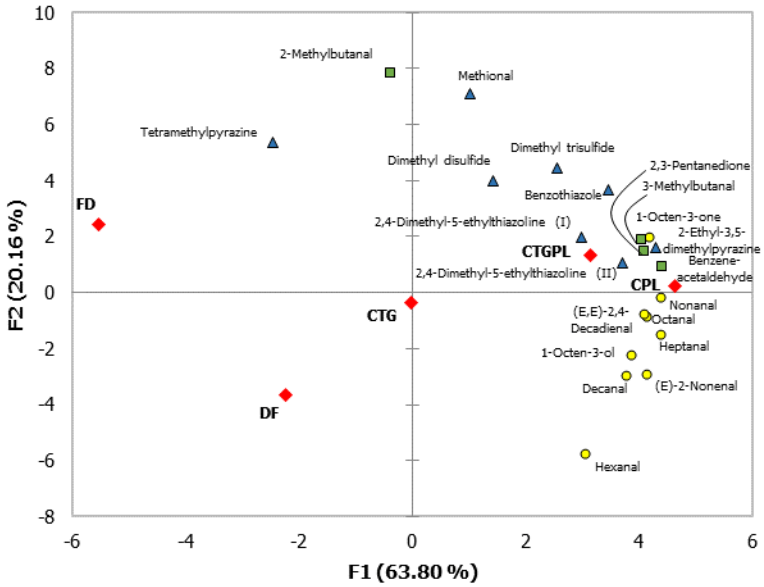


Figure 3-6: Principal component analysis of (reconstituted) samples (FMM = frozen minced meat sample; FD = freeze-dried meat sample; DF = defatted meat sample containing medium chain triglyceride; CTG = defatted meat sample containing chicken triglycerides; CPL = defatted meat sample containing chicken phospholipids; CTGPL = defatted meat sample containing chicken triglycerides and phospholipids in 1:1 w/w ratio) showing correlations with odour-active volatiles identified in LN aroma extracts (□: Maillard reaction product; ∆: S-/N-/O-containing compound; •: lipid degradation product)

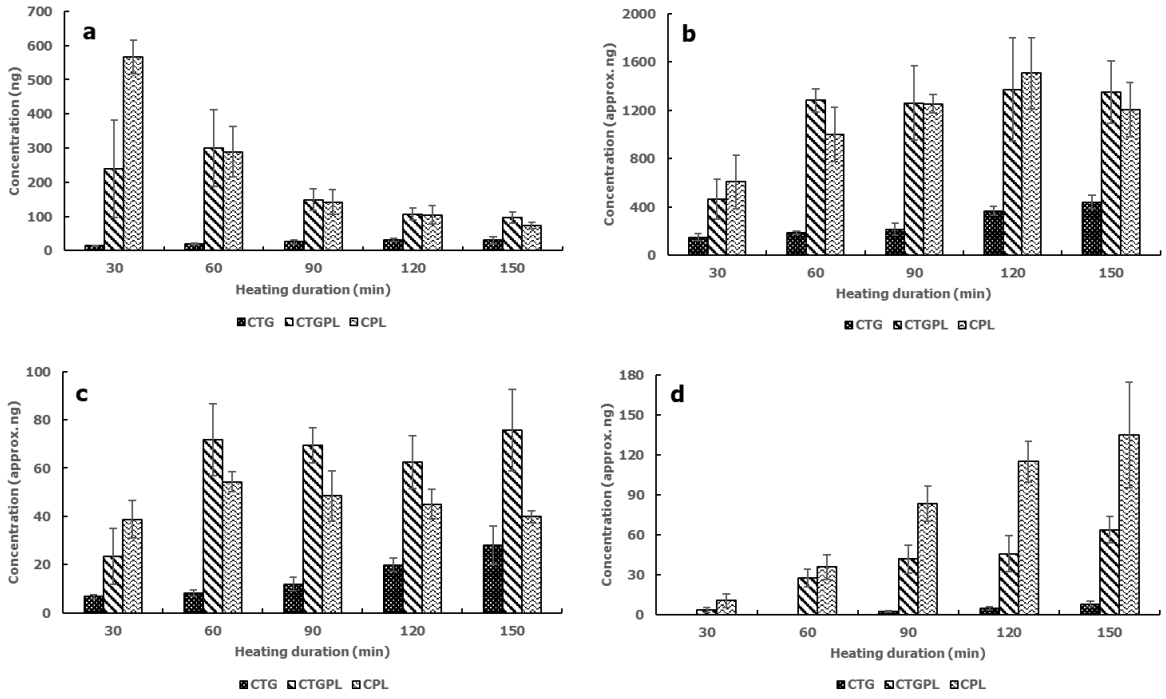
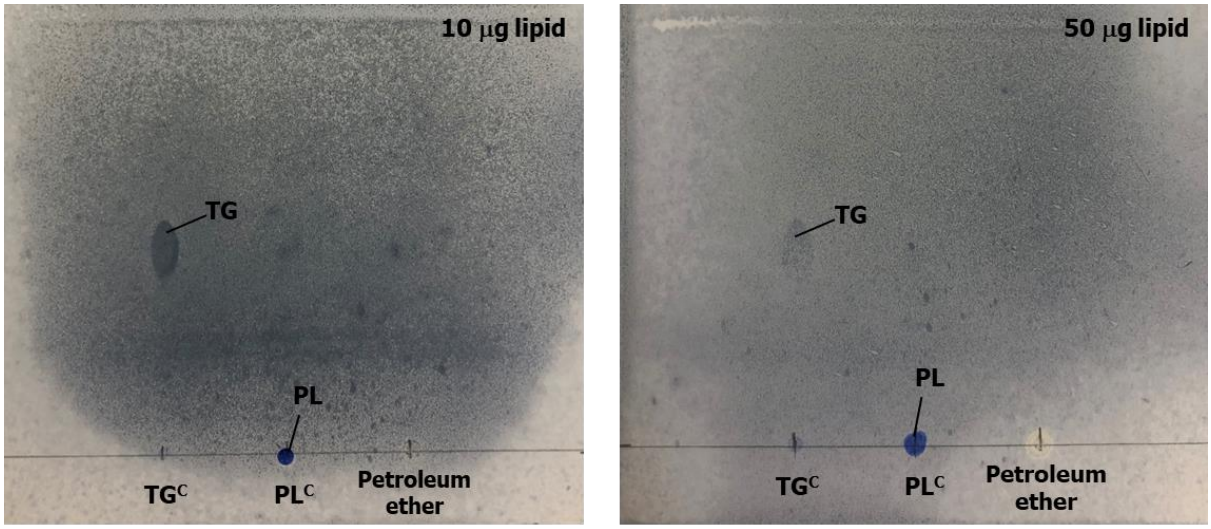


Figure 3-7: Mean quantities (ng or approx. ng) of a) (E,E)-2,4-decadienal b) hexanal c) (E)-2-octenal and d) (E,Z)-2,4-nonadienal in the headspace of reconstituted samples (CTG=

defatted meat sample containing chicken triglycerides; CTGPL= defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w); CPL= defatted meat sample containing chicken phospholipids; n=4 with error bars representing standard deviation of replicates)

3.7 Appendices

Appendix A: Thin layer chromatography of purified chicken triglyceride (TG^C) and chicken phospholipid (PL^C) eluted with hexane: diethyl ether:formic acid (80:20:2 v/v)



Appendix B: Ion transitions and instrument settings for amino acid detection using LC-MS/MS

Amino acid	Precursor (m/z)	Product (m/z)	Frag (V)	CE (V)	RT (min)
Arginine	175.1	70.1	92	24	8.6
	175.1	60.1	92	12	8.6
Asparagine	133.1	87.1	78	4	7.3
	133.1	74.1	78	12	7.3
Aspartic acid	134	88.1	64	4	7.3
	134	74	64	8	7.3
Glutamine	147.1	130.1	64	4	7.3
	147.1	84.1	64	12	7.3
Glutamic acid	148	130	50	4	6.7
	148	84.1	50	12	6.7
Glycine	76	30	50	4	7.3
Histidine	156.1	110.1	92	8	8.5
	156.1	83.1	92	20	8.5
Ileucine	132.1	69.1	60	16	5.3
Leucine	132.1	86.1	60	4	5.3
	132.1	44.1	60	20	5.3
Lysine	147.1	130	92	4	8.8
	147.1	84.1	92	16	8.8
Methionine	150	133	64	4	5.5
	150	56.1	64	16	5.5
Phenylalanine	166.1	120.1	64	8	4.8
	166.1	103.1	64	20	4.8
Proline	116	70.1	64	16	6.3
Serine	106	60.1	64	8	7.3

	106	42.1	64	20	7.3
Threonine	120	74	50	8	6.9
	120	56.1	50	16	6.9
Tryptophan	205.1	188.1	78	4	5.0
	205.1	146.1	78	20	5.0
Tyrosine	182.1	165	50	4	5.7
	182.1	136.1	50	8	5.7
Valine	118.1	72.1	64	4	6.0
	118.1	55.2	64	20	6.0
Alanine	90.1	44.2	64	8	6.9

Frag = Fragmentor voltage; CE = Collision energy; RT = Retention time

Appendix C: Quantification ions and relative response factors used in the semi-quantification of compounds in the preliminary experiment

Compound	**LRI**	**Quant ion**	**RRF**
Hexanal	800	44	7.17
1-Hexanol	872	56	3.83
2-Heptanone	892	43	2.45
Heptanal	902	70	10.2
3-(Methylthio)propanal	908	104	11.9
1-Octen-3-ol	981	57	2.86
2,3-Octanedione	984	43	2.06
2-Pentylfuran	994	81	2.26
Octanal	1004	43	8.73
2-Phenylacetaldehyde	1053	91	2.22
(E)-2-Octenal	1062	55	11.0
1-Octanol	1070	56	7.69
4-Nonanone (IS)	1074	43	4.25
Nonanal	1106	57	8.99
(E)-2-Nonenal	1164	41	11.9
Decanal	1207	43	13.6
(E)-2-Decenal	1265	70	13.1
Undecanal	1308	41	17.0
(E,E)-2,4-Decadienal	1322	152	92.3
Tridecanal	1512	57	9.11
Tetradecanal	1615	57	13.8
Pentadecanal	1720	82	14.1
Hexadecanal	1824	82	13.6
(Z)-13-Octadecenal	2012	55	9.58
Octadecanal	2031	43	10.8

LRI= Linear retention indices determined on DB-5 column; Quant ion= Quantification ion; RRF= Relative response factor; IS= Internal standard

Appendix D: Quantification ions and relative response factors used in the semi-quantification of compounds in the LN-SDE experiment

Compound	LRI	Quant ion	RRF
3-Methylbutanal	657	58	7.50
2-Methylbutanal	664	58	8.35
2,3-Pentanedione	696	100	23.9
Dimethyl disulfide	746	94	3.27
1-Pentanol	778	70	11.2
Hexanal	802	82	33.1
(E)-2-Hexenal	864	69	9.84
2-Heptanone	891	43	2.57
Heptanal	903	70	11.2
3-(Methylthio)propanal	912	48	4.88
(E)-2-Heptenal	963	68	28.6
2-Isopropylpyrazine (IS)	974	107	3.55
1-Octen-3-one	978	70	4.77
1-Octen-3-ol	983	72	15.1
Dimethyl trisulfide	984	126	3.98
2,3-Octanedione	988	71	8.74
2-Pentylfuran	992	138	12.8
2-Octanone	992	128	37.2
(E)-2-(2-Pentenyl)furan	1001	136	11.0
Octanal	1007	84	18.0
2-Phenylacetaldehyde	1058	91	2.05
(E)-2-Octenal	1063	83	18.9
(E)-2-Octen-1-ol	1071	55	17.0
1-Octanol	1073	84	15.8
4-Nonanone (IS)	1074	99	15.4
5-Ethyl-2,4-dimethyl-3-thiazoline (I)	1080	143	16.9
2-Ethyl-3,5-dimethylpyrazine	1084	135	2.73
Tetramethylpyrazine	1091	54	3.41
2-Nonanone	1093	58	4.51
5-Ethyl-2,4-dimethyl-3-thiazoline (II)	1099	143	18.4
Nonanal	1107	57	9.34
(E)-3-Nonen-2-one	1144	125	9.80
(E)-2-Nonenal	1168	70	12.1
1-Nonanol	1173	83	24.0
2-Decanone	1194	58	3.33
Decanal	1209	55	16.8
Benzothiazole	1235	135	2.94
(E)-2-Decenal	1266	70	14.0
2-Undecanone	1293	58	3.55
(E,E)-2,4-Decadienal	1333	81	3.86
Tetradecanal	1618	57	14.0
Pentadecanal	1715	57	11.7
Hexadecanal	1818	57	14.7

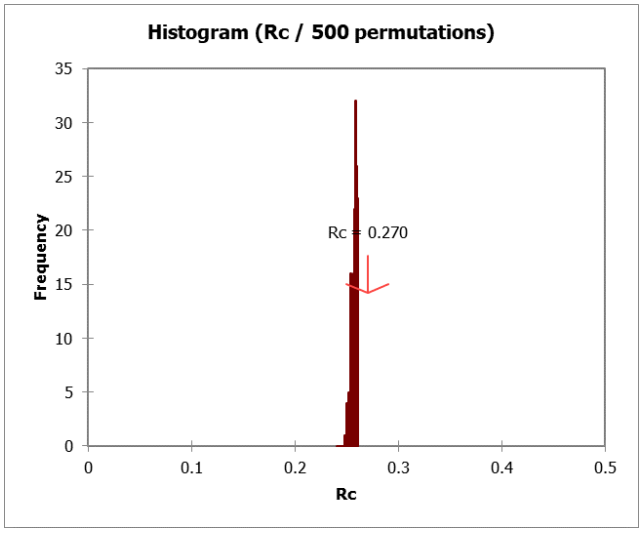
LRI= Linear retention indices determined on DB-5 column; Quant ion= Quantification ion; RRF= Relative response factor; IS= Internal standard

Appendix E: Quantification ions and relative response factors used in the semi-quantification of compounds in the kinetics experiment

Compound	LRI	Quant ion	RRF
Hexanal	800	44	7.41
1-Hexanol	869	56	4.77
2-Heptanone	891	43	2.73
Heptanal	902	70	8.90
1-Octen-3-ol	979	57	2.81
2,3-Octanedione	982	43	2.62
2-Pentylfuran	994	81	2.31
Octanal	1004	43	11.2
(E)-2-Octenal	1061	55	10.4
1-Octanol	1070	56	8.85
4-Nonanone (IS)	1075	43	4.47
Nonanal	1106	57	8.75
(E)-2-Nonenal	1164	41	13.1
(E,Z)-2,4-Nonadienal	1197	81	2.86
Decanal	1208	43	13.9
Undecanal	1310	41	13.7
(E,E)-2,4-Decadienal	1323	152	29.6
Dodecanal	1411	57	11.7
Tridecanal	1515	57	12.1
Tetradecanal	1618	57	12.2
Pentadecanal	1721	82	13.5
Hexadecanal	1825	82	12.7
(Z)-13-Octadecenal	2012	55	14.1
Octadecanal	2032	43	16.6

LRI= Linear retention indices determined on DB-5 column; Quant ion= Quantification ion; RRF= Relative response factor; IS= Internal standard

Appendix F: Results of consensus test in Generalised Procrustes Analysis of sensory data



Permutations	500
Rc	0.270
Quantile	74.600

The consensus test is a permutation test which determines whether the observed Rc value (Rc corresponds to the proportion of the original variance explained by the consensus configuration) is significantly higher than 95% of the results that are obtained when permuting the data. As the Rc value is away from the set of random results on the histogram, it indicates that the consensus configuration obtained is a true solution.

4. Effect of sunflower and soy lipids on boiled chicken aroma generation

Abstract

The substitution of animal lipids with plant lipids in plant-based meat products poses technical challenges, such as flavour generation to retain the meaty aroma characteristics, due to the inherent differences between lipids from different sources. Chicken, sunflower and soy triglycerides and phospholipids were added individually or as a mixture (1:1 w/w) to a defatted chicken meat matrix and the aroma generated during heating was extracted using Likens-Nickerson simultaneous distillation-extraction (LN-SDE) for analysis by GC-MS. The results showed that the incorporation of phospholipids in the samples significantly enhanced the production of some S- and/or N-containing Maillard reaction products and most of the lipid-derived products. Meanwhile, the effect of lipid source was less clear and seemed to be dependent on the degree of unsaturation in the fatty acids as well as the class of phospholipids. Besides the lower saturated fatty acid content, plant phospholipids were found to be twice as high in polyunsaturated:monounsaturated fatty acid ratio as compared to chicken phospholipids. Nevertheless, sunflower phospholipids proved to be a better choice than the other lipids studied as a substitution for chicken phospholipids despite the higher concentration of n-6 fatty acids and lower concentration of n-3 and n-9 fatty acids in the former.

Keywords: Sunflower, soy, lipid oxidation, phospholipid, chicken aroma

4.1 Introduction

The shift in consumer landscape towards vegan, vegetarian and flexitarian diets, driven by mounting environmental, animal welfare and health concerns over industrial animal agriculture (He et al., 2020), has led to a surge in demand for plant-based meat (PBM) products over the last decade. The new generation PBM aims to emulate the appearance, taste, texture, consumer experience and nutritional profile of meat but is made from plant-sourced protein and other ingredients (Specht, 2018). However, the inherent differences between animal muscle and plant materials present inevitable technological hurdles to be overcome.

While intensive efforts have been made in the research and development of alternative proteins, less attention has been placed on the replacement of animal fats with vegetable oils. Fat, as an essential component of meat and meat products, contributes to important quality attributes such as flavour, texture, mouthfeel, appearance, emulsion stability and cooking yield (Jiménez Colmenero, 2000). Unlike animal fats which are usually high in saturated fatty acids (SFAs) and cholesterol (Valsta et al., 2005), vegetable oils are high in unsaturated fatty acids (UFAs)and free from cholesterol. While the latter are a healthier choice from a nutritional standpoint, the differences in physicochemical properties, such as fatty acid (FA) composition, flavour and colour, pose technical challenges for the replication of meat-like sensory characteristics.

Variations in FA composition not only affect the firmness or softness of the fat in meat at both the inter- and intra- muscular level, but also the appearance as solidified fats with a higher melting point are observed to be whiter than that with a lower melting point (Wood et al., 2003). Furthermore, UFAs have a higher propensity to undergo oxidation, which contributes to flavour development during cooking, as well as rancidity and colour deterioration over the shelf-life of the product.

Although the replacement of animal fat with vegetable oils in reduced-fat meat products for improved nutrition and organoleptic properties is now commonplace (Choi et al., 2010; Shin et al., 2020; Youssef & Barbut, 2011), most of the investigation within the PBM sector to date has been directed towards the structuring of edible oils through organogelation to mimic the texture of animal fat (Marangoni & Edmund Daniel, 2012) rather than from a flavour perspective. Yet, flavour was found to be an important criteria for the successful adoption of meat substitutes by consumers (Weinrich, 2019). Not only do lipids serve as important precursors for the formation of odour-active lipid degradation products (Mottram, 1998), but also provide a hydrophobic phase for the reaction and deposit of these molecules (Frérot, 2017). In addition, lipids influence flavour perception through phase partitioning and mass transport effects, as well as contribute to texture and mouthfeel (de Roos, 2005). Lipids which are currently used in PBM include sunflower, soybean, rapeseed, canola, corn, palm and coconut oil (Kyriakopoulou et al., 2019). Among these, soybean and sunflower are amongst the most important oilseed crops in the world with their global production reaching 366 and 49 million metric tons respectively in 2021 (USDA, 2021).

The aim of this study is to investigate the effects of different sources and fractions of plant lipids, namely sunflower and soybean triglycerides and phospholipids, on boiled chicken (BC) aroma generation using a defatted meat matrix to achieve a system as close to real meat as possible. The hypothesis is that plant phospholipids could be used as a substitution for chicken phospholipids since they both have a high unsaturated fatty acid content.

4.2 Materials and methods

4.2.1 Materials and chemicals

Aroma chemicals were obtained from the following suppliers and were ≥95% in purity unless stated otherwise: hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, tridecanal, 3-(methylthio)propanal, 2-methylbutanal, (E)-2-octenal, (E)-2-nonenal, (E)-2-hexenal, 1-octanol, 1-nonanol, 1-octen-3-ol, (E)-2-octen-1-ol, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, 2-undecanone, 1-octen-3-one, (E)-3-nonen-2-one, 2,3-pentanedione, 2,3-octanedione, dimethyl disulfide, dimethyl trisulfide, benzothiazole, tetramethylpyrazine and 2-isopropylpyrazine from Sigma Aldrich (Gillingham, UK); 2-ethyl-3,5-dimethylpyrazine from Fluorochem (Hadfield, UK); 3-methylbutanal from Alfa Aeser (Heysham, UK); 2-phenylacetaldehyde from Acros Organics (New Jersey, USA); 2-pentylfuran from Avocado Research Chemicals (London, UK); (E)-2-decenal, (E)-2-heptenal from Fluka (Seelze, Germany); (E,E)-2,4-decadienal (90%) from Lancaster Synthesis (Heysham, UK); and 1-pentanol, 4-nonanone from TCI (Oxford, UK).

Diethyl ether, pentane (98%), petroleum ether (boiling point 40 – 60 °C; ≥75%), isooctane, 0.5 M sodium methoxide solution in methanol (0.5 N), sodium chloride, methyl tricosanoate and Supelco 37 Component FAME Mix (CRM47885) were from Sigma-Aldrich. Chloroform and methanol were from Fisher Scientific (Loughborough, UK). Florisil® (30 – 60 mesh) was from VWR Chemicals (Lutterworth, UK). All the chemicals were ≥99% in purity unless specified otherwise. High purity water (18.2 MΩ) was obtained from a Select Fusion Ultrapure water deionisation unit (SUEZ, Peterborough, UK) for instrumental analysis.

Sunflower triglyceride (TG^{SF}) was from KTC (Wednesbury, Sandwell, UK), soy triglyercide (TG^{SY}) was from Haepyo (Sajo Haepyo, Seoul, Korea) while sunflower phospholipid (PL^{SF}; LEC5636) and soy phospholipid (PL^{SY}; LeciTAs® 5348) were from Thew Arnott (Deeside, Flintshire, UK). Once removed from the original packaging, all lipids were transferred to amber

glass bottles and stored under nitrogen at -20 °C. Phospholipids were dissolved in a small amount of chloroform, which was removed under nitrogen prior to usage. Fresh Class A chicken breasts were purchased from a retail supermarket and processed within the sell-by date. They were a standard Ross 308 genotype provided by one commercial poultry supplier and were from the same batch within an experiment.

4.2.2 Chicken lipid extraction and purification

The chicken breasts were trimmed of extramuscular fat, minced, freeze-dried, ground, thoroughly mixed and vacuum packed in aluminium pouches and stored at -80 °C prior to use. A 2-stage Soxhlet lipid extraction was carried out: first, using petroleum ether followed by chloroform/methanol (2:1 v/v) to obtain a chicken triglyceride (TG^C) and chicken phospholipid (PL^C) fraction respectively. Both stages were carried out at 50 – 60 °C for 8 and 6 cycles respectively before the solvent was removed by rotary evaporation at 40 °C. The lipid extracts and meat fraction were kept under vacuum overnight to remove residual solvent. The TG^C extract was purified using Florisil following the procedure of Farmer and Mottram (1990) while the PL^C extract was washed using 0.2 times the volume of Ultrapure water (Folch et al., 1957) before dissolution in chloroform for storage. All lipid extracts were stored under nitrogen at -20 °C while the defatted meat was vacuum packed in aluminium pouches and stored at -80 °C.

TG^C and PL^C extracts were tested for contamination using TLC on silica gel 60 plates (Supelco, Merck Millipore) eluted with hexane: diethyl ether: formic acid (80:20:2 v/v). Different amounts (10 and 50 μg) of each extract were applied to the TLC plate to estimate the level of impurities. The spots were visualised using a phosphorous spray (Appendix G). As the purpose of the purification procedure was to obtain a lipid sample in sufficient quantities for characterisation and experiments, and not to obtain an accurate lipid compositional analysis for the muscle, this degree of separation was considered adequate.

4.2.3 Sample preparation

The composition of the samples is listed in Table 4-1. An equal part of water was added to facilitate mixing and for sample heating. All the samples were prepared in 1 L round bottom flasks and the reconstituted samples were flushed under nitrogen for 5 min, stoppered, mixed using a magnetic stirrer until the lipid was incorporated and stored at 4 °C overnight for equilibration. An internal standard mix of 2-isopropylpyrazine and 4-nonanone (both 10 ng μL^{-1} in 100 μL methanol) was added to the sample the following day before extraction. A total of 4 replicates were prepared for each treatment (TG= Defatted meat sample containing triglycerides; TGPL= Defatted meat sample containing triglycerides and phospholipids (1:1 w/w); PL= Defatted meat sample containing phospholipids; whereby the prefix before C, SF and SY represent the source of lipid chicken, sunflower and soy respectively).

4.2.4 Likens-Nickerson simultaneous distillation-extraction

Each 25 g of reconstituted sample was boiled in an equal part of water at 100 °C in a heating mantle for 30 min. LN-SDE was performed using 30 mL of redistilled pentane: diethyl ether (9:1 v/v) for 2 h. The extract was concentrated to 0.5 mL using a Vigreux column, flushed under a gentle stream of N_2 to 0.1 mL and stored at -80 °C prior to analysis. A total of 4 replicates were prepared for each treatment.

4.2.5 GC-MS analysis of volatile compounds

GC-MS analyses were performed on an Agilent 6890N GC equipped with an Agilent 5975 inert XL EI/CI triple axis mass spectroscopy detector (Agilent Technologies, Santa Clara, CA, USA). An aliquot of sample (1 µL) was injected in a pulsed splitless mode (pulse pressure 18.5 psi; 0.5 min). Chromatographic separation was achieved on a HP-5 MS column (30 m x 0.25 mm x 1 μm, Agilent Technologies). The oven temperature was increased from 35 °C (held for 10

min) to 200 °C at 6 °C min^{-1} and to 320 °C at 15 °C min^{-1} (held for 10 min). The carrier gas was helium at a constant flow rate of 1.2 mL min^{-1}. The MS was operated in electron impact mode with a source temperature of 230 °C, ionisation energy of 70 eV and a scan range from m/z 20 to m/z 400.

A series of C_5 – C_{25} *n*-alkanes was analysed under the same conditions for the calculation of the linear retention index (LRI) of each compound. The identities of the compounds were confirmed based on a match of their mass spectra and LRI with those of authentic compounds where available. Otherwise, a tentative identification was made by comparing their mass spectra against the NIST 14 library and LRI available in literature. 2-isopropylpyrazine was used as the internal standard for S- and/or N-containing compounds while 4-nonanone was used as the internal standard for the other compounds. Semi-quantification of the compounds was performed by comparison of their respective peak areas with that of the internal standards using relative response factors (RRFs). The quantification ions and RRFs used are listed in Appendix **H**.

4.2.6 Fatty acid analysis

Fatty acid methyl ester (FAME) derivatisation was carried out using an adapted method from Bannon et al. (1982). Briefly, 1 mL of diethyl ether containing 1 mg mL^{-1} tricosanoic acid methyl ester as internal standard and 2 mL of 0.5 M sodium methoxide solution in methanol was added to 50 mg of lipid before stirring for 5 min at 1000 rpm. This was followed by the addition of 2 mL of isooctane and 5 mL of saturated sodium chloride solution before vigorously shaking at 1800 rpm. Upon separation, the upper layer was removed for analysis.

FAME separation was performed on an Agilent 7890B GC coupled to a flame ionisation detector (Agilent Technologies) using a HP-88 column (100 m x 0.25 mm x 0.2 μm; Agilent

Technologies). The injection volume was 1 µL at a 50:1 split ratio. The oven temperature was increased from 120 °C (held for 1 min) to 175 °C at 10 °C min^{-1} (held for 10 min), then to 210 °C at 5 °C min^{-1} (held for 5 min), and finally to 230 °C at 5 °C min^{-1} (held for 5 min). The carrier gas was hydrogen at a constant flow rate of 1.5 mL min^{-1}. The temperature of the injector and detector were 250 °C and 280 °C respectively. Individual FAMEs were identified by comparing their retention times with those of a standard 37 component FAME mix. Quantification of the compounds was performed by comparison of their respective peak areas against that of the internal standards. A total of 3 replicates were carried out and results were expressed as percentage of total FAs (%).

4.2.7 Statistical analysis

All statistical analyses were carried out using XLSTAT v2021.4.1 (Addinsoft Inc, Paris, France). Two-way analysis of variance (ANOVA),followed by Fisher's least square significant difference (LSD) post-hoc tests at a significance level of $p = 0.05$, were used to analyse the main and interaction effects of lipid class and source on the quantity of volatile measured. Principal component analysis (PCA) was performed to visualise the differences observed between the samples using the odour-active compounds identified as variables.

4.3 Results and discussion

4.3.1 Fatty acid composition of lipids

The predominant FAs in all the lipids were C16:0 (palmitic), C18:0 (stearic), C18:1 (oleic) and C18:2 (linoleic), albeit in varying proportions, and contributed to ≥ 80% of total FA content. The values in Table 4-2 were in close agreement with literature data for chicken lipids (Ferioli & Caboni, 2010; Jahan & Paterson, 2007; Marion & Woodroof, 1965; Sahasrabudhe et al., 1985) and plant lipids (Carelli et al., 2002; Doert et al., 2012; Farhoosh et al., 2009; Orsavova et al., 2015; van Nieuwenhuyzen & Tomás, 2008; Velasco & Fernández-Martínez, 2002). A

graphical representation of the different FA classes, namely saturated fatty acid (SFA), monounsaturated fatty acid n-9 (MUFA n-9), polyunsaturated fatty acid n-3 (PUFA n-3), PUFA n-6, is provided in Figure 4-1.

The chicken lipids had a higher SFA content of 29 – 39 % as compared to plant lipids which ranged from 12 – 28 %. This could be attributed to the widespread industry practice of fat supplementation in poultry diet to increase the energy density and feed efficiency to promote the growth and performance of broiler chickens, with the aim of achieving the highest meat yield in the shortest time (Ravindran et al., 2016). The choice of fat used is largely driven by cost during commercial production and palm oil is commonly used as it is one of the cheapest edible oils in the world (Carter et al., 2007). Studies had shown that the utilisation of palm oil in diets led to an increase in SFA content, such as C14:0 (myristic) and C16:0, and higher SFA:UFA enhanced meat firmness, which was beneficial for marketing (Smink et al., 2008; Valencia et al., 1993).

On the other hand, the plant lipids were richer in PUFA content ranging from 56 – 59%, which was approximately twice that of chicken lipids at 27 – 36%. In addition, although all the PLs had a higher PUFA:MUFA ratio compared to the TGs from the same source, the ratio was at least twice as high in PL^{SF} and PL^{SY} at 3.3 and 3.6 respectively as compared to PL^{C} at 1.5. This could be due to the selective and intensive breeding of high-value crop cultivars with improved nutritional quality and functional properties for use in refined edible oil applications. Elevated PUFA content is a desirable trait for oil crops due to the increased consumer health awareness and dietary recommendations for the limitation of SFA intake in conjunction with PUFA replacement (FAO, 2010) based on scientific evidence that PUFAs confer cardioprotective effects on human health while SFAs on the contrary, are notorious for their association with risk factors of cardiovascular diseases (Baum et al., 2012). In addition, C18:2 and C18:3 are

essential to the human diet as they cannot be synthesised *de novo* and are required for the production of eicosanoids which participate in inflammatory and immune responses (Patterson et al., 2012).

Among the C18 UFAs, C18:2(n-6) was present at similarly high concentrations of 50 – 60% across all 4 plant lipids, followed by C18:1(n-9) with a range of 15 – 31%, while C18:3(n-3) was present at much lower levels in soy lipids at around 5% and not present in sunflower lipids. Besides the abovementioned role of FAs, C18 UFAs also fulfill specific modulatory functions in plant pathogen defence. They contribute to the biosynthesis of plant cuticular components, which form a physical barrier against fungal pathogens (Kachroo & Kachroo, 2009), as well as the production of oxylipins, which are metabolites from the oxidative transformation of UFAs and confer resistance against biotic and abiotic stresses (Blée, 2002). Although C18:3 is responsible for the synthesis of jasmonic acid, which is a major oxylipin hormone regulating plant defense and development (Blée, 2002), it is also the unstable precursor of secondary lipid oxidation products, which cause the beany, grassy odour note of 'reversion' flavour in oils (Dutton et al., 1951; Ullrich & Grosch, 1988). Thus, oilseed cultivars with a low level of C18:3 were preferentially developed to increase the oxidative stability of the edible oils (Rahman & Takagi, 1997), thereby extending product shelf life and expanding food applications. Furthermore, the FA composition of edible oils could be influenced by plant genotype, environmental conditions and agricultural practices (Linder, 2000; Schulte et al., 2013; Werteker et al., 2010), as well as extraction techniques used during industrial processing (Farhoosh et al., 2009; Kasote et al., 2013; Pradhan et al., 2010; Smiles et al., 1988).

Last but not least, the presence of *trans*-fatty acid (TFA) was almost non-existent in the lipids studied. Due to worldwide initiatives to eliminate industrially produced TFA, edible oils are

extremely low in TFA, meaning <2 g per 100 g of fat according to EU regulation 2019/649 (European Commission, 2019). However, very low quantities (1 – 2%) could still be produced during conventional deodorisation, which is the final but crucial step of refining to produce an oil with light colour, neutral taste and good oxidative stability, as the high temperature treatment, often above 200 °C, could lead to the geometric isomerisation of PUFAs (Liu et al., 2021). As for the poultry lipids, the level of TFA is naturally low as poultry birds could only produce *t*9,*t*12-C18:2 via endogenous synthesis unlike ruminants which could undergo microbial biohydrogenation of PUFAs (Schmid et al., 2006).

4.3.2 Effect of lipid class (TG and PL) on volatile generation

4.3.2.1 Lipid-derived products

Table 4-3 lists the key volatiles identified in the LN aroma extracts. Significant differences in the abundance of lipid-derived products were observed between samples which were reconstituted with lipids from the same source (e.g. TG^{C}, PL^{C} and $TGPL^{C}$). In general, quantities of the majority of the lipid-derived volatiles in the samples increased in the following order: TG < TGPL < PL samples across all 3 sources (e.g. CTG < CTGPL < CPL). This trend could be observed in Figure 4-2 which illustrates some of the lipid-derived volatiles.

For the n-6 and n-3 lipid-derived volatiles, this could be attributed to the significantly higher composition of the corresponding FAs present in the PLs as compared to TGs, except for PL^{SY} which had a similar level of n-6 FAs as TG^{SY} at around 51% with no significant difference between both and a 1% lower level of n-3 FAs which was small but significant. There was a significantly higher concentration of C18:2(n-6) and C18:3(n-3) in PL^{SF} as compared to TG^{SF} while in the case of PL^{C}, a significantly higher proportion of n-6 and n-3 FAs with longer carbon chains (≥20) and more sites of unsaturation (≥2 C=C double bonds), such as C20:5n-3 (1%), C22:6n-3 (0.8%), C20:4n-6 (7.2%) and C22:2n-6 (0.4%) with the difference listed in brackets,

would lead to increased oxidative susceptibility and higher reactivity (Dominguez et al., 2019) as explained in greater detail in Chapter 3, Section 3.3.4.1.

Although there was a significantly lower concentration of n-9 FAs in all PLs as compared to TGs, there were significantly more n-9 lipid-derived compounds, namely octanal, nonanal, decanal, 1-octanol, 1-nonanol, found in TG(PL) samples as compared to TG samples across all sources as shown in Table 4-3. Since all the n-9 FAs are MUFAs, the difference in reactivity of the FAs would not be related to their degree of unsaturation and was more likely due to the influence of the lipid structure. It was reported that TGs with UFA in the *sn*-2 position demonstrated higher oxidative stability than those with UFA in the *sn*-1(3) (Wada & Koizumi, 1983; Wijesundera et al., 2008) and was attributed to the variation in physical orientation of the FA in different positions and the interaction with the hydroperoxides (Ghnimi et al., 2017). Moreover, once the initial stage of lipid oxidation had taken place, leading to the formation of alkyl radicals from highly unsaturated PUFAs such as those in PLs, the accumulation of alkyl radicals could promote the oxidation and degradation of the less reactive FAs such as the MUFAs, thereby accelerating the subsequent propagation steps in the lipid oxidation chain reaction (Elmore et al., 1999).

4.3.2.2 Maillard reaction products

The Maillard reaction between reducing sugars and amino compounds is important for the generation of highly reactive intermediates, such as dicarbonyls, which can participate in an array of reactions with hydrogen sulfide and ammonia for the formation of odour-active compounds. S- and/or N-containing compounds are especially potent due to their low odour threshold values. These include but are not limited to furanthiols, mercaptoketones, sulfides, thiazoles, thiazolines, pyrroles, pyridines and pyrazines (Mottram, 1998). Only 3-thiazolines and pyrazines were found in this study, which was likely due to the presence of the other

compounds at concentrations too low for GC-MS detection. Meanwhile, the Strecker degradation of amino acids (an associated reaction of the Maillard reaction) yields aldehydes such as 3-methylbutanal, 2-methylbutanal, 3-(methylthio)propanal and 2-phenylacetaldehyde identified in this study. However, not all Maillard reaction products were affected by the addition of different classes of lipids.

Significant differences in the abundance of some S- and/or N-containing compounds were found between samples which were reconstituted with TGs and/or PLs within the same source (i.e. chicken, sunflower or soy). As seen in Figure 4-3 for the pair of 5-ethyl-2,4-dimethyl-3-thiazoline isomers, there was on average twice the amount present in (TG)PL samples as compared to TG samples across all the sources. 3-Thiazolines were reported to possess nutty, roasted, meaty, onion and vegetable-like odours, depending on the substituents (Elmore et al., 1997; Mussinan et al., 1976) and could be formed from the reaction of α-hydroxyketones or α-dicarbonyls with hydrogen sulfide and ammonia in the presence of aliphatic aldehydes (Elmore & Mottram, 1997). In particular, 5-ethyl-2,4-dimethyl-3-thiazoline has a savoury, grilled meat, fatty and juicy character as reported in Chapter 2 and could be formed from 3-mercapto-2-pentanone and acetaldehyde. 3-Mercapto-2-petanone is a Maillard reaction product which could be formed from the reaction between 4-hydroxy-5-methyl-3(2H)-furanone and cysteine via the α-diketone pathway (Cerny & Davidek, 2003), or between 1,4-dideoxyosone of ribose (5-hydroxy-2,3-pentanedione) and hydrogen sulfide (originating from cysteine) (Cerny & Davidek, 2003), or thiamin degradation (Guentert et al., 1990). Meanwhile, acetaldehyde could be generated via the Strecker degradation of amino acids such as cysteine and alanine (Rizzi, 2008), as well as the degradation of reactive 2-alkenals and 2,4-alkadienals such as 2-octenal and 2,4-decadienal (Zamora et al., 2015). Since the acetaldehyde precursor could be influenced by lipid oxidation, it was likely that the higher rate of lipid oxidation in the presence of PLs would promote the

formation of 5-ethyl-2,4-dimethylthiazoline and thus, resulted in a significantly higher abundance of this compound in both forms of isomer found in the (TG)PL samples. Such a lack of reactive precursors in TG as compared to PL for the interaction and absorption of reactive free molecules, and consequently a reduction in the formation of long chain heterocycles was also observed by Farmer and Mottram (1990) in model systems.

This finding was in contrast to that reported for thiazoles, a closely related class of compounds, whereby the addition of lecithin had no marked changes in its qualitative composition (Whitfield et al., 1988) and neither did the class of lipid have a significant effect on its formation (Farmer & Mottram, 1990). However, thiazoles are the oxidation products of thiazolines and a direct comparison may not be the most appropriate but thiazolines are less widely reported due to their susceptibility to oxidation and the harsher reaction conditions employed in the studies on model systems (≥140 °C for at least an hour) would favour the formation of thiazoles. On the other hand, milder conditions were used in this work to mimic the preparation of stocks or casseroles in a kitchen setting, partly explaining the observation of 3-thiazolines instead of alkylthiazoles, although the presence of the latter group of compounds was plausible as previously reported in Chapter 2 but their abundance in these extracts of a much smaller sample size would be too low for detection by GC-MS.

Significantly higher quantities of pyrazines were also found in samples containing (TG)PL as compared to TG. As observed in Figure 4-3, there was about twice the amount of tetramethylpyrazine in (TG)PL samples of chicken or soy origin as compared to TG samples, whereas a smaller magnitude difference of 1.25 times was observed in 2-ethyl-3,5-dimethylpyrazine between C(TG)PL and CTG samples only. This was in contrast to findings in model systems of cysteine and ribose, whereby the lack of an effect of PL addition was attributed to the formation of aminoketones, which are the precursors of pyrazine formation,

as minor products of the Strecker degradation of cysteine in an acidic medium (pH 5.6) (Farmer & Mottram, 1990; Whitfield et al., 1988). On the other hand, Mottram and Edwards (1983) observed a reduction of methylpyrazine and dimethylpyrazine in reconstituted lean beef extracts in the presence of PLs but not TGs. However, 2-ethyl-3,5-dimethylpyrazine and tetramethylpyrazine were not reported in their study and the extent of any inhibition exerted by the lipids or its degradation products on these compounds is unknown. In addition, the yield of pyrazine formation is also dependent on the type of amino acids present, with lysine being one of the most efficient (Hwang et al., 1995). It was reported in Chapter 2 that lysine was one of the most abundant amino acids in the DF meat matrix and this could also have an impact on how the lipids influence pyrazine formation in the system.

4.3.3 Effect of lipid source (chicken, sunflower and soy) on volatile generation

Unlike lipid class, the effect of lipid source on volatile generation was more obscure and the degree of unsaturation in the FAs as well as the class of PL seemed to be crucial factors. Among the lipid-derived volatiles, the trend for the n-9 derived lipid oxidation products was more straightforward, whereby a significantly higher proportion of n-9 FAs in PL^{C} than PL^{SF} and PL^{SY} generally corresponded with higher quantities of these lipid-derived volatiles in the CPL samples as compared to SFPL and SYPL samples. However, such a distinction was not observed for the TG samples despite the significantly higher composition of TG^{C} than TG^{SF} and TG^{SY}.

Although all the plant lipids were dominated by n-6 FAs (>50%), with at least twice the concentration of C18:2n-6 as compared to the chicken lipids, only some n-6 derived lipid oxidation products were present in significantly higher abundance in samples containing plant lipids as compared to those containing chicken lipids. For these compounds, such as hexanal and heptanal in the SFPL samples and (E)-2-heptenal in SFPL and SYPL samples as compared

to CPL samples, as well as (E,E)-2,4-decadienal, (E)-2-heptenal and (E)-2-decenal in SFTG samples compared to CTG samples, the observations could naturally be explained by the higher concentration of n-6 FAs present. However, 1-octen-3-one, (E)-2-nonenal, 1-octen-3-ol, (E)-2-octen-1-ol, (E)-2-nonenal and (E)-2-decenal were present in significantly higher quantities in the CPL samples as compared to SFPL and SYPL samples. This could be due to the presence of more reactive n-6 FAs with longer carbon chains (≥20) and more sites of unsaturation (≥2) present in PL^{C}, namely C20:2, C20:3 and C20:4, which were either not found or present in extremely low quantities in PL^{SF} and PL^{SY}. Similarly for the n-3 lipid derived volatiles, the higher quantity of (E)-(2-pentenyl)furan observed in C(TG)PL samples as compared to SY(TG)PL samples could be explained by the presence of the more reactive C20:5n-3 in PL^{C}, which was not found in PL^{SY}, despite the higher proportion of n-3 FAs present in the latter. In addition, there could be the possibility of a higher trace of native antioxidants, such as tocopherols and polyphenols, present in the plant lipids as compared to animal lipids (Choe & Min, 2006) although industrial refining processes, such as deodourisation, could lead to a reduction in antioxidants (Szydłowska-Czerniak & Łaszewska, 2015). Nevertheless, plant lecithins have been shown to exhibit protective effects against oxidation due to a synergistic effect between the amino-alcohol group of PLs and tocopherols (Doert et al., 2012; Judde et al., 2003).

Besides the FA composition, the PL class composition should also be considered as the different functional head groups could confer distinct properties on the lipid molecule. As seen in Table 4-4, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the major classes across all PLs. In addition, sphingomyelin (SM) is only found in animals but not in higher plants due to the different enzymes present in the metabolic pathways (Tafesse et al., 2006). Although PL class composition could vary depending on factors such as the means of solvent extraction, as well as chemical and enzymatic modification (van Nieuwenhuyzen &

Tomás, 2008), PL^{SF} generally has a higher concentration of PC and PI than PL^{SY}, which has a slightly higher concentration of PE (Monakhova & Diehl, 2016; Nguyen et al., 2014).

The different chemical modifications to the phosphate head group of the PLs could have an impact on the reactions with the other components in the system. PE was reported to offer an enhanced protective effect in liposomal dispersions as result of the formation of antioxidative, hydrophobic pyrroles from reactions between the primary amino group in PE and carbonyls in lipid oxidation products (Goritschnig et al., 2020; Lu et al., 2013). In addition, it was demonstrated that PE could regenerate α-tocopherol through amino-carbonyl reactions but PC lacked the ability to do so due to its quaternary amino group (Doert et al., 2012), which instead contributed to the reduction in antioxidative capacity of PC upon oxidation (Hidalgo et al., 2005). Meanwhile, the antioxidative effect of PA was attributed to the suppression of the catalytic activity of non-haem iron and hemoprotein (Dacaranhe & Terao, 2001). Based on the compiled literature data in Table 4-4, although the level of PE was within the same range across the lipids, there would likely be a higher proportion of PC in PL^{C} while PA would only be present in the plant lipids. Thus, it could be possible that PL^{C} had a higher reactivity than the other lipids in the system, although the antioxidative capacity of PLs is dependent on a myriad of factors such as the existence of other antioxidants in the system which could have a synergistic effect, prooxidants which could catalyse the rate of lipid oxidation, and even the positional distribution of FA in the lipid molecule.

4.3.4 Lipid class-source interactions and PCA

When considering lipid class-source interactions, it could be observed from **Error! Reference source not found.** that in general when there was a lack of impact of either lipid class or source, the interaction would usually be insignificant too. For the majority of the Maillard reaction products, there were no significant lipid-source interactions, which could be attributed

to the lack of significant impact of lipid class on the abundance of these compounds and the weak impact of lipid source. This was in contrast to the majority of the lipid-derived products, where significant lipid class-source interactions were found ($p < 0.001$). This was inevitable since lipid class characteristics such as FA composition are dependent on its source.

To investigate which plant lipid (combination) would be the most suitable replacement for chicken lipids, the data on odour-active volatiles is visually represented using PCA plot as shown in Figure 4-4. Principal components 1 (PC1), 2 (PC2) and 3 (PC3) accounted for 49.7%, 24.4% and 10.9% of the total variation within the data respectively. The first axis separated the samples containing any of the TGs (i.e. CTG, SFTG and SYTG samples) and those containing soy phospholipids (i.e. SYPL and SYTGPL samples), the second axis separated the samples containing any of the plant PLs (i.e. SFPL, SFTGPL, SYPL and SYTGPL samples), while the third axis separated the samples containing PL^{C} or PL^{SF} (i.e. CPL, CTGPL, SFPL and SFTGPL samples). The samples containing any of the TGs and $(TG^{SY})PL^{SY}$ and those with $(TG^{C})PL^{C}$ or $(TG^{SF})PL^{SF}$ were displayed as opposites with the former group exhibiting a stronger correlation with (E,E)-2,4-decadienal and hexanal while the latter group was more closely associated with a balanced mix of Maillard reaction and lipid oxidation products. The closer relationship demonstrated between SF(TG)PL and C(TG)PL samples suggests that PL^{SF} is a more befitting alternative for PL^{C} than the other lipids studied in this work. However, the SF(TG)PL samples were still different from the C(TG)PL target as there was an overall higher quantity of odour-active lipid-derived volatiles, such as hexanal, heptanal and (E,E)-2,4-decadienal, but lower quantity of some Maillard reaction products, such as dimethyl disulfide, dimethyl trisulfide and 2-ethyl-3,5-dimethylpyrazine, in the former. The addition of antioxidants (e.g. tocopherols) could be a possible solution to retard the rate of formation of lipid oxidation products, thereby increasing the availability of precursors for the Maillard reaction to take place.

4.4 Conclusion

Lipid oxidation during meat aroma generation is a multifactorial phenomenon in a system which is essentially a thermodynamically unstable oil-in-water emulsion. Thus, intrinsic differences such as FA composition, lipid class and source had an impact on the extent of the lipid oxidation, as well as exerted some influence on the Maillard reaction as observed in the higher quantity of S- and N-containing compounds produced in systems containing PLs as compared to TGs. However, the replacement of chicken lipids with plant lipids is not as simplistic as matching FA composition or lipid class types but also taking into consideration the presence of other minor components, such as native antioxidants or prooxidants, which might naturally be present in these lipids. Nevertheless, this work suggested that PL^{SF} has potential for use in the substitution of chicken lipids in plant-based meat products.

4.5 References

Balazs, P. E., Schmit, P. L., & Szuhaj, B. F. (1996). High-performance liquid chromatographic separations of soy phospholipids. *Journal of the American Oil Chemists' Society, 73*(2), 193-197.

Bannon, C. D., Breen, G. J., Craske, J. D., Hai, N. T., Harper, N. L., & O'Rourke, K. L. (1982). Analysis of fatty acid methyl esters with high accuracy and reliability: III. Literature review of and investigations into the development of rapid procedures for the methoxide-catalysed methanolysis of fats and oils. *Journal of Chromatography A, 247*(1), 71-89.

Baum, S. J., Kris-Etherton, P. M., Willett, W. C., et al. (2012). Fatty acids in cardiovascular health and disease: A comprehensive update. *Journal of Clinical Lipidology, 6*(3), 216-234.

Blée, E. (2002). Impact of phyto-oxylipins in plant defense. *Trends in Plant Science, 7*(7), 315-322.

Carelli, A. A., Ceci, L. N., & Crapiste, G. H. (2002). Phosphorus-to-phospholipid conversion factors for crude and degummed sunflower oils. *Journal of the American Oil Chemists' Society, 79*(12), 1177-1180.

Carter, C., Finley, W., Fry, J., Jackson, D., & Willis, L. (2007). Palm oil markets and future supply. *European Journal of Lipid Science and Technology, 109*(4), 307-314.

Cerny, C., & Davidek, T. (2003). Formation of aroma compounds from ribose and cysteine during the maillard reaction. *Journal of Agricultural and Food Chemistry, 51*(9), 2714-2721.

Chapman, G. W. (1980). A conversion factor to determine phospholipid content in soybean and sunflower crude oils. *Journal of the American Oil Chemists' Society, 57*(9), 299-302.

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive reviews in food science and food safety, 5*(4), 169-186.

Choi, Y. S., Choi, J. H., Han, D. J., et al. (2010). Effects of replacing pork back fat with vegetable oils and rice bran fiber on the quality of reduced-fat frankfurters. *Meat Science, 84*(3), 557-563.

Dacaranhe, C. D., & Terao, J. (2001). Effect of phosphatidic acid and phosphatidylserine on lipid oxidation in beef homogenate during storage and in emulsified sardine oil. *Journal of Food Science, 66*(3), 422-427.

de Roos, K. B. (2005). How lipids influence flavor perception. In *Food Lipids* (Vol. 920, pp. 145-158): American Chemical Society.

Doert, M., Jaworska, K., Moersel, J. T., & Kroh, L. W. (2012). Synergistic effect of lecithins for tocopherols: Lecithin-based regeneration of α-tocopherol. *European Food Research and Technology, 235*(5), 915-928.

Dominguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants (Basel), 8*(10), 429.

Dutton, H. J., Lancaster, C. R., Evans, C. D., & Cowan, J. C. (1951). The flavor problem of soybean oil. VIII. Linolenic acid. *Journal of the American Oil Chemists' Society, 28*(3), 115-118.

Elmore, J. S., & Mottram, D. S. (1997). Investigation of the reaction between ammonium sulfide, aldehydes, and α-hydroxyketones or α-dicarbonyls to form some lipid-Maillard interaction products found in cooked beef. *Journal of Agricultural and Food Chemistry, 45*(9), 3595-3602.

Elmore, J. S., Mottram, D. S., Enser, M., & Wood, J. D. (1997). Novel thiazoles and 3-thiazolines in cooked beef aroma. *Journal of Agricultural and Food Chemistry, 45*(9), 3603-3607.

Elmore, J. S., Mottram, D. S., Enser, M., & Wood, J. D. (1999). Effect of the polyunsaturated fatty acid composition of beef muscle on the profile of aroma volatiles. *Journal of Agricultural and Food Chemistry, 47*(4), 1619-1625.

Commission Regulation (EU) 2019/649 of 24 April 2019 amending Annex III to Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards trans fat, other than trans fat naturally occurring in fat of animal origin, (EU) 2019/649 C.F.R. (2019).

FAO. (2010). *Fats and fatty acids in human nutrition. Report of an expert consultation* (0254-4725). Retrieved from https://www.fao.org/3/i1953e/I1953E.pdf

Farhoosh, R., Einafshar, S., & Sharayei, P. (2009). The effect of commercial refining steps on the rancidity measures of soybean and canola oils. *Food Chemistry, 115*(3), 933-938.

Farmer, L. J., & Mottram, D. S. (1990). Interaction of lipid in the maillard reaction between cysteine and ribose: The effect of a triglyceride and three phospholipids on the volatile products. *Journal of the Science of Food and Agriculture, 53*(4), 505-525.

Ferioli, F., & Caboni, M. F. (2010). Composition of phospholipid fraction in raw chicken meat and pre-cooked chicken patties: influence of feeding fat sources and processing technology. *European Food Research and Technology, 231*(1), 117-126.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry, 226*(1), 497-509.

Frérot, E. (2017). Fats and oils. In A. Buettner (Ed.), *Springer Handbook of Odor* (pp. 223-259). Cham, Switzerland: Springer.

Ghnimi, S., Budilarto, E., & Kamal-Eldin, A. (2017). The new paradigm for lipid oxidation and insights to microencapsulation of omega-3 fatty acids. *Comprehensive reviews in food science and food safety, 16*(6), 1206-1218.

Goritschnig, J., Tadus, K., König, J., & Pignitter, M. (2020). Free radical scavenging activity of carbonyl-amine adducts formed in soybean oil fortified with phosphatidylethanolamine. *Molecules, 25*(2), 373.

Guentert, M., Bruening, J., Emberger, R., et al. (1990). Identification and formation of some selected sulfur-containing flavor compounds in various meat model systems. *Journal of Agricultural and Food Chemistry, 38*(11), 2027-2041.

He, J., Evans, N. M., Liu, H., & Shao, S. (2020). A review of research on plant-based meat alternatives: Driving forces, history, manufacturing, and consumer attitudes. *Comprehensive reviews in food science and food safety, 19*(5), 2639-2656.

Hidalgo, F. J., Nogales, F., & Zamora, R. (2005). Changes produced in the antioxidative activity of phospholipids as a consequence of their oxidation. *Journal of Agricultural and Food Chemistry, 53*(3), 659-662.

Hwang, H. I., Hartman, T. G., & Ho, C. T. (1995). Relative reactivities of amino acids in pyrazine formation. *Journal of Agricultural and Food Chemistry, 43*(1), 179-184.

Jahan, K., & Paterson, A. (2007). Lipid composition of retailed organic, free‐range and conventional chicken breasts. *International Journal of Food Science & Technology, 42*(3), 251-262.

Jiménez Colmenero, F. (2000). Relevant factors in strategies for fat reduction in meat products. *Trends in Food Science & Technology, 11*(2), 56-66.

Judde, A., Villeneuve, P., Rossignol-Castera, A., & Le Guillou, A. (2003). Antioxidant effect of soy lecithins on vegetable oil stability and their synergism with tocopherols. *Journal of the American Oil Chemists' Society, 80*(12), 1209-1215.

Kachroo, A., & Kachroo, P. (2009). Fatty Acid–Derived Signals in Plant Defense. *Annual Review of Phytopathology, 47*(1), 153-176.

Kasote, D. M., Badhe, Y. S., & Hegde, M. V. (2013). Effect of mechanical press oil extraction processing on quality of linseed oil. *Industrial Crops and Products, 42*, 10-13.

Kyriakopoulou, K., Dekkers, B., & van der Goot, A. J. (2019). Plant-Based Meat Analogues. In C. M. Galanakis (Ed.), *Sustainable Meat Production and Processing* (pp. 103-126): Academic Press.

Linder, C. R. (2000). Adaptive Evolution of Seed Oils in Plants: Accounting for the Biogeographic Distribution of Saturated and Unsaturated Fatty Acids in Seed Oils. *The American Naturalist, 156*(4), 442-458.

Liu, X., Zhou, S., Jiang, Y., & Xu, X. (2021). Optimization of deodorization design for four different kinds of vegetable oil in industrial trial to reduce thermal deterioration of product. *Journal of the American Oil Chemists' Society, 98*(4), 475-483.

Lončarević, I., Pajin, B., Petrović, J., et al. (2016). The impact of sunflower and rapeseed lecithin on the rheological properties of spreadable cocoa cream. *Journal of Food Engineering, 171*, 67-77.

Lu, F. S. H., Nielsen, N. S., Baron, C. P., Diehl, B. W. K., & Jacobsen, C. (2013). Impact of primary amine group from aminophospholipids and amino acids on marine phospholipids stability: Non-enzymatic browning and lipid oxidation. *Food Chemistry, 141*(2), 879-888.

Marangoni, A. G., & Edmund Daniel, C. (2012). Organogels: An alternative edible oil-structuring method. *Journal of the American Oil Chemists' Society, 89*(5), 749-780.

Marion, J., & Miller, W. (1968). Phospholipids and Component Fatty Acids of Chicken Tissues. *Poultry Science, 47*, 1453-1459.

Marion, J. E., & Woodroof, J. G. (1965). Lipid fractions of chicken broiler tissues and their fatty acid composition. *Journal of Food Science, 30*(1), 38-43.

Monakhova, Y. B., & Diehl, B. W. K. (2016). Quantitative Analysis of Sunflower Lecithin Adulteration with Soy Species by NMR Spectroscopy and PLS Regression. *Journal of the American Oil Chemists' Society, 93*(1), 27-36.

Mottram, D. S. (1998). Flavour formation in meat and meat products: A review. *Food Chemistry, 62*(4), 415-424.

Mottram, D. S., & Edwards, R. A. (1983). The role of triglycerides and phospholipids in the aroma of cooked beef. *Journal of the Science of Food and Agriculture, 34*(5), 517-522.

Mussinan, C. J., Wilson, R. A., Katz, I. R. A., Hruza, A., & Vock, M. H. (1976). Identification and flavor properties of some 3-oxazolines and 3-thiazolines isolated from cooked beef. In *Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors* (Vol. 26, pp. 133-145): American Chemical Society.

Nguyen, M. T., Van de Walle, D., Petit, C., Beheydt, B., Depypere, F., & Dewettinck, K. (2014). Mapping the chemical variability of vegetable lecithins. *Journal of the American Oil Chemists' Society, 91*(7), 1093-1101.

Orsavova, J., Misurcova, L., Ambrozova, J. V., Vicha, R., & Mlcek, J. (2015). Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. *International Journal of Molecular Sciences, 16*(6), 12871-12890.

Patterson, E., Wall, R., Fitzgerald, G. F., Ross, R. P., & Stanton, C. (2012). Health implications of high dietary omega-6 polyunsaturated Fatty acids. *Journal of nutrition and metabolism, 2012*, 539426-539426.

Peng, C. Y., & Dugan Jr., L. R. (1965). Composition and structure of phospholipids in chicken muscle tissues. *Journal of the American Oil Chemists' Society, 42*(6), 533-536.

Pradhan, R. C., Meda, V., Rout, P. K., Naik, S., & Dalai, A. K. (2010). Supercritical CO2 extraction of fatty oil from flaxseed and comparison with screw press expression and solvent extraction processes. *Journal of Food Engineering, 98*(4), 393-397.

Rahman, S. M., & Takagi, Y. (1997). Inheritance of reduced linolenic acid content in soybean seed oil. *Theoretical and Applied Genetics, 94*(3), 299-302.

Ravindran, V., Tancharoenrat, P., Zaefarian, F., & Ravindran, G. (2016). Fats in poultry nutrition: Digestive physiology and factors influencing their utilisation. *Animal Feed Science and Technology, 213*, 1-21.

Rizzi, G. P. (2008). The strecker degradation of amino acids: Newer avenues for flavor formation. *Food Reviews International, 24*(4), 416-435.

Sahasrabudhe, M. R., Delorme, N. F., Wood, D. F., & Randall, C. J. (1985). Neutral and polar lipids in chicken parts and their fatty acid composition. *Poultry Science, 64*(5), 910-916.

Schmid, A., Collomb, M., Sieber, R., & Bee, G. (2006). Conjugated linoleic acid in meat and meat products: A review. *Meat Science, 73*(1), 29-41.

Schulte, L. R., Ballard, T., Samarakoon, T., et al. (2013). Increased growing temperature reduces content of polyunsaturated fatty acids in four oilseed crops. *Industrial Crops and Products, 51*, 212-219.

Shin, D.-J., Lee, H. J., Lee, D., Jo, C., & Choe, J. (2020). Fat replacement in chicken sausages manufactured with broiler and old laying hens by different vegetable oils. *Poultry Science, 99*(5), 2811-2818.

Smiles, A., Kakuda, Y., & MacDonald, B. E. (1988). Effect of degumming reagents on the recovery and nature of lecithins from crude canola, soybean and sunflower oils. *Journal of the American Oil Chemists' Society, 65*(7), 1151-1155.

Smink, W., Gerrits, W. J. J., Hovenier, R., et al. (2008). Fatty Acid Digestion and Deposition in Broiler Chickens Fed Diets Containing Either Native or Randomized Palm Oil. *Poultry Science, 87*(3), 506-513.

Specht, L. (2018). Meat by the molecule: making meat with plants and cells. *Biochem (Lond), 40*(4), 18-21.

Szydłowska-Czerniak, A., & Łaszewska, A. (2015). Effect of refining process on antioxidant capacity, total phenolics and prooxidants contents in rapeseed oils. *LWT - Food Science and Technology, 64*(2), 853-859.

Tafesse, F. G., Ternes, P., & Holthuis, J. C. M. (2006). The multigenic sphingomyelin synthase family. *Journal of Biological Chemistry, 281*(40), 29421-29425.

Ullrich, F., & Grosch, W. (1988). Flavour Deterioration of Soya-bean Oil: Identification of Intense Odour Compounds Formed during Flavour Reversion. *Lipid / Fett, 90*(9), 332-336.

USDA. (2021). *Major Oilseeds: World Supply and Distribution (Commodity View)*. Retrieved from https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf

Valencia, M. E., Watkins, S. E., Waldroup, A. L., Waldroup, P. W., & Fletcher, D. L. (1993). Utilization of Crude and Refined Palm and Palm Kernel Oils in Broiler Diets. *Poultry Science, 72*(12), 2200-2215.

Valsta, L. M., Tapanainen, H., & Männistö, S. (2005). Meat fats in nutrition. *Meat Science, 70*(3), 525-530.

van Nieuwenhuyzen, W., & Tomás, M. C. (2008). Update on vegetable lecithin and phospholipid technologies. *European Journal of Lipid Science and Technology, 110*(5), 472-486.

Velasco, L., & Fernández-Martínez, J. M. (2002). Breeding oilseed crops for improved oil quality. *Journal of Crop Production, 5*(1-2), 309-344.

Wada, S., & Koizumi, C. (1983). Influence of the position of unsaturated fatty acid esterified glycerol on the oxidation rate of triglyceride. *Journal of the American Oil Chemists' Society, 60*(6), 1105-1109.

Weinrich, R. (2019). Opportunities for the Adoption of Health-Based Sustainable Dietary Patterns: A Review on Consumer Research of Meat Substitutes. *Sustainability, 11*(15), 4028.

Werteker, M., Lorenz, A., Johannes, H., Berghofer, E., & Findlay, C. S. (2010). Environmental and Varietal Influences on the Fatty Acid Composition of Rapeseed, Soybeans and Sunflowers. *Journal of Agronomy and Crop Science, 196*(1), 20-27.

Whitfield, F. B., Mottram, D. S., Brock, S., Puckey, D. J., & Salter, L. J. (1988). Effect of phospholipid on the formation of volatile heterocyclic compounds in heated aqueous solutions of amino acids and ribose. *Journal of the Science of Food and Agriculture, 42*(3), 261-272.

Wijesundera, C., Ceccato, C., Watkins, P., et al. (2008). Docosahexaenoic acid is more stable to oxidation when located at the sn-2 position of triacylglycerol compared to sn-1(3). *Journal of the American Oil Chemists' Society, 85*(6), 543-548.

Wood, J. D., Richardson, R. I., Nute, G. R., et al. (2003). Effects of fatty acids on meat quality: a review. *Meat Science, 66*(1), 21-32.

Youssef, M. K., & Barbut, S. (2011). Fat reduction in comminuted meat products-effects of beef fat, regular and pre-emulsified canola oil. *Meat Science, 87*(4), 356-360.

Zamora, R., Navarro, J. L., Aguilar, I., & Hidalgo, F. J. (2015). Lipid-derived aldehyde degradation under thermal conditions. *Food Chemistry, 174*, 89-96.

4.6 Tables and figures

4.6.1 Tables

Table 4-1: Composition of reconstituted samples for LN-SDE experiment

Component	Weight in reconstituted samples (g)		
	(C/SF/SY)TG	(C/SF/SY)TGPL	(C/SF/SY)PL
Freeze-dried meat	-	-	-
Defatted meat	5.8	5.8	5.8
$TG^{C/SF/SY}$	0.5	0.25	-
$PL^{C/SF/SY}$	-	0.25	0.5
Water	18.7	18.7	18.7

$TG^{C/SF/SY}$= Triglycerides from chicken/ sunflower/ soy; **$PL^{C/SF/SY}$**= Phospholipids from chicken/ sunflower/ soy; (**C/SF/SY)TG**= Defatted meat sample containing chicken/ sunflower/ soy triglycerides; **(C/SF/SY)TGPL**= Defatted meat sample containing chicken/ sunflower/ soy triglycerides and phospholipids (1:1 w/w); **(C/SF/SY)PL**= Defatted meat sample containing chicken/ sunflower/ soy phospholipids

Table 4-2: Fatty acid composition in lipids (% of measured fatty acids; n=3)

Fatty acid	TG^{C}	PL^{C}	TG^{SF}	PL^{SF}	TG^{SY}	PL^{SY}	SEM[1]	p[2]
SFA								
C10:0	0.03^{b}	0.10^{c}	$n.d.^{a}$	$n.d.^{a}$	$n.d.^{a}$	$n.d.^{a}$	0.01	***
C12:0	0.03^{d}	$n.d.^{a}$	$n.d.^{b}$	0.01^{c}	$n.d.^{b}$	0.01^{b}		
C14:0	0.51^{c}	0.35^{b}	0.08^{a}	0.09^{a}	0.08^{a}	0.09^{a}	0.04	***
C15:0	0.09^{e}	0.13^{f}	0.02^{b}	0.06^{c}	$n.d.^{a}$	0.08^{d}	0.01	***
C16:0	22.1^{d}	26.2^{f}	6.55^{a}	17.2^{c}	11.3^{b}	23.2^{e}	1.68	***
C17:0	0.13^{b}	0.19^{c}	0.04^{a}	0.16^{bc}	0.06^{a}	0.14^{b}	0.01	***
C18:0	5.93^{e}	11.1^{f}	3.77^{b}	4.39^{d}	3.90^{c}	3.47^{a}	0.64	***
C20:0	0.08^{b}	0.04^{a}	0.28^{d}	0.29^{d}	0.42^{e}	0.16^{c}	0.03	***
C21:0	0.02^{a}	0.24^{b}	0.01^{a}	0.07^{a}	0.04^{a}	0.04^{a}	0.02	**
C22:0	0.03^{b}	$n.d.^{a}$	0.80^{e}	0.90^{f}	0.50^{d}	0.40^{c}	0.08	***
C24:0	0.02^{a}	0.12^{b}	0.27^{d}	0.25^{cd}	0.18^{bc}	0.28^{d}	0.02	***
MUFA								
C16:1n-7	4.33^{d}	1.22^{c}	0.12^{ab}	0.08^{a}	0.09^{a}	0.14^{b}	0.37	***
C14:1n-9	0.13^{c}	$n.d.^{a}$	$n.d.^{a}$	$n.d.^{a}$	0.02^{b}	$n.d.^{a}$	0.01	***
t9-C18:1	0.21^{d}	0.13^{c}	0.03^{b}	0.03^{b}	$n.d.^{a}$	0.02^{b}	0.02	***
c9-C18:1	38.4^{f}	21.7^{c}	30.5^{e}	17.4^{b}	25.8^{d}	15.6^{a}	1.90	***
C20:1n-9	0.44^{e}	0.36^{d}	0.17^{b}	0.09^{a}	0.25^{c}	0.08^{a}	0.03	***
C22:1n-9	0.03^{d}	$n.d.^{a}$	0.01^{b}	0.02^{c}	0.01^{b}	$n.d.^{a}$	0.20	***
C24:1n-9	0.01^{a}	1.60^{b}	0.01^{a}	0.06^{a}	$n.d.^{a}$	$n.d.^{a}$	0.14	***
PUFA								
C18:3n-3	3.07^{c}	1.69^{b}	$n.d.^{a}$	0.18^{a}	5.53^{e}	4.75^{d}	0.51	***
C20:3n-3	0.05^{c}	0.28^{d}	$n.d.^{a}$	$n.d.^{a}$	0.01^{b}	0.02^{b}	0.02	***
C20:5n-3	0.07^{a}	1.02^{b}	0.01^{a}	0.01^{a}	0.02^{a}	$n.d.^{a}$	0.09	***

Fatty acid	TG^{C}	PL^{C}	TG^{SF}	PL^{SF}	TG^{SY}	PL^{SY}	SEM[1]	p[2]
C22:6n-3	0.06^{a}	0.83^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.08	***
t9,t12- C18:2	0.03^{ab}	n.d.^{a}	0.01^{a}	0.05^{b}	n.d.^{a}	0.01^{a}	0.01	*
c9,c12- C18:2	23.0^{b}	21.8^{a}	57.3^{d}	58.6^{e}	51.8^{c}	51.5^{c}	3.76	***
C18:3n-6	0.19^{c}	0.10^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.02	***
C20:2n-6	0.24^{b}	1.13^{c}	0.01^{a}	0.01^{a}	0.04^{a}	0.03^{a}	0.10	***
C20:3n-6	0.22^{b}	1.42^{c}	n.d.^{a}	n.d.^{a}	0.01^{a}	n.d.^{a}	0.13	***
C20:4n-6	0.47^{b}	7.65^{c}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.68	***
C22:2n-6	n.d.^{a}	0.36^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	0.03	***
Total								
SFA	29.0^{b}	38.5^{a}	11.8^{f}	23.4^{d}	16.5^{e}	27.9^{c}	2.11	***
MUFA	43.6^{a}	25.0^{d}	30.8^{b}	17.7^{e}	26.2^{c}	15.8^{f}	2.22	***
PUFA	27.4^{e}	36.3^{d}	57.3^{b}	58.9^{a}	57.4^{b}	56.3^{c}	3.01	***
n-3	3.24^{d}	3.82^{c}	0.01^{e}	0.19^{e}	5.55^{a}	4.77^{b}	0.52	***
n-6	24.1^{e}	32.5^{d}	57.3^{b}	58.7^{a}	51.8^{c}	51.5^{c}	3.16	***
n-9	39.2^{a}	23.8^{d}	30.7^{b}	17.6^{e}	26.0^{c}	15.7^{f}	1.92	***

TG^{C}= Chicken triglyceride; **PL^{C}**= Chicken phospholipid; **TG^{SF}**= Sunflower triglyceride; **PL^{SF}**= Sunflower phospholipid; **TG^{SY}**= Soy triglyceride; **PL^{SY}**= Soy phospholipid

SFA= C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C24:0; **MUFA**= C14:1, C16:1n-7, t9-C18:1, c9-C18:1, C20:1n-9, C22:1n-9, C24:1n-9; **PUFA**= t9,t12-C18:2, c9,c12-C18:2, C18:3n-3, C18:3n-6, C20:2n-6, C20:3n-3, C20:3n-6, C20:4n-6, C20:5n-3, C22:2n-6, C22:6n-3

^{a,b,c,d,e} Means with the same superscript letters are not significantly different (p = 0.05) as determined by Fisher's LSD test; n.d.= not detected

[1] Standard error mean

[2] Probability that there is a significant difference between means as determined by Fisher's LSD test; *, ** and *** denote significant differences at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ respectively

Table 4-3: Mean quantities (approx. ng) of the key volatile compounds identified in the LN aroma extracts (n=4)

Compound	LRI[1]	ID[2]	Mean quantities of volatiles (approx. ng)[3]									SEM[4]	p[5]		
			CTG	CTGPL	CPL	SFTG	SFTGPL	SFPL	SYTG	SYTGPL	SYPL		C	S	CxS
Maillard reaction products															
3-Methylbutanal†	657	A	54.3^{ab}	53.8^{ab}	73.8^{a}	27.1^{c}	52.7^{b}	46.6^{bc}	34.6^{bc}	34.2^{bc}	34.3^{bc}	3.15	n.s	**	n.s
2-Methylbutanal†	664	A	180^{abc}	219^{a}	183^{abc}	129^{c}	205^{ab}	195^{abc}	151^{bc}	160^{abc}	170^{abc}	8.12	n.s	n.s	n.s
2,3-Pentanedione†	696	A	426^{a}	484^{a}	436^{a}	276^{b}	417^{a}	374^{ab}	369^{ab}	374^{ab}	378^{ab}	16.2	n.s	*	n.s
Dimethyl disulfide†	746	A	774^{ab}	1030^{a}	655^{ab}	712^{ab}	363^{b}	446^{b}	674^{ab}	851^{ab}	1020^{a}	62.9	n.s	*	n.s
3-(Methylthio)propanal†	912	A	252^{ab}	239^{ab}	272^{a}	186^{ab}	288^{a}	174^{ab}	178^{ab}	224^{ab}	158^{b}	13.8	n.s	n.s	n.s
Dimethyl trisulfide†	984	A	1450^{abc}	2120^{a}	1430^{abc}	1300^{abc}	717^{c}	860^{bc}	1230^{abc}	1740^{ab}	1870^{a}	121	n.s	*	n.s
2-Phenylacetaldehyde†	1058	A	2040^{abc}	2450^{ab}	2660^{a}	1630^{c}	2210^{abc}	1800^{bc}	1960^{abc}	1820^{bc}	1840^{bc}	88.5	n.s	*	n.s
5-Ethyl-2,4-dimethyl-3-thiazoline (I)†	1080	A	28.2^{c}	45.2^{abc}	63.6^{a}	35.5^{bc}	61.0^{a}	60.7^{a}	29.1^{c}	50.6^{abc}	52.7^{ab}	3.21	**	n.s	n.s
2-Ethyl-3,5-dimethylpyrazine†	1081	A	275^{b}	342^{a}	346^{a}	142^{c}	179^{c}	162^{c}	153^{c}	183^{c}	161^{c}	14.1	*	***	n.s
Tetramethylpyrazine†	1091	A	70.9^{cd}	99.3^{bc}	144^{a}	61.6^{d}	111^{ab}	126^{ab}	61.4^{d}	94.5^{bcd}	69.8^{cd}	5.86	***	*	*
5-Ethyl-2,4-dimethyl-3-thiazoline (II)†	1099	A	54.3^{c}	95.7^{ab}	107^{a}	57.2^{bc}	119^{a}	119^{a}	48.9^{c}	121^{a}	119^{a}	6.45	***	n.s	n.s
Benzothiazole†	1235	A	113^{c}	307^{b}	480^{a}	18.3^{d}	n.d.^{d}	32.6^{d}	n.d.^{d}	n.d.^{d}	n.d.^{d}	27.9	***	***	***
n-3 lipid derived															
1-Pentanol	778	A	88.7^{e}	118de	277^{cd}	165de	483^{b}	758^{a}	111de	752^{a}	392^{bc}	46.1	***	***	***
(E)-2-Hexenal	864	A	12.9^{bcd}	16.7^{bc}	33.1^{a}	16.3^{bc}	n.d.^{d}	3.57^{cd}	7.28^{cd}	42.5^{a}	27.3^{ab}	2.73	n.s	**	**
(E)-2-(2-pentenyl)furan	1001	A	9.91^{c}	31.9^{b}	47.8^{a}	14.0^{c}	20.0^{c}	13.0^{c}	14.4^{c}	17.3^{c}	14.8^{c}	2.24	*	***	**
n-6 lipid derived															
Hexanal†	802	A	3870^{d}	3570^{d}	4760^{bcd}	5120^{abcd}	6100^{abc}	6750^{a}	4800^{bcd}	6300^{ab}	4490^{cd}	249	n.s	*	n.s
Heptanal†	903	A	581^{ef}	725de	870^{bcd}	511^{f}	913^{bc}	1290^{a}	506^{f}	755^{cd}	1010^{b}	44.1	***	*	*
(E)-2-Heptenal	963	A	271^{c}	313^{c}	410^{c}	811^{ab}	815^{ab}	772^{ab}	303^{c}	833^{a}	664^{b}	42.4	**	***	**
1-Octen-3-one†	978	A	34.0^{bc}	40.4^{b}	50.6^{a}	33.1^{bc}	27.3^{cd}	24.8^{d}	31.2^{cd}	28.6^{cd}	29.2^{cd}	1.50	n.s	***	*
1-Octen-3-ol†	983	A	709^{b}	776^{b}	993^{a}	692^{b}	491^{c}	443^{c}	708^{b}	512^{c}	495^{c}	31.5	*	***	***
2-Pentylfuran	992	A	425^{e}	733^{bcde}	1110^{a}	522de	933^{ab}	887^{abc}	554^{cde}	821^{abcd}	604^{bcde}	50.0	*	n.s	n.s
(E)-2-Octenal	1063	A	679^{c}	837^{bc}	1180^{a}	1000^{ab}	1070^{ab}	1170^{a}	836^{bc}	976^{ab}	978^{ab}	36.9	*	*	n.s
(E)-2-Octen-1-ol	1071	A	63.8^{c}	151^{b}	296^{a}	74.7^{c}	87.5^{c}	82.1^{c}	49.0^{c}	69.3^{c}	62.0^{c}	13.3	***	***	***

Compound	LRI[1]	ID[2]	Mean quantities of volatiles (approx. ng)[3]									SEM[4]	p[5]		
			CTG	CTGPL	CPL	SFTG	SFTGPL	SFPL	SYTG	SYTGPL	SYPL		C	S	CxS
(E)-3-Nonen-2-one	1144	A	9.85^{c}	24.7^{b}	37.7^{a}	9.91^{c}	27.3^{b}	44.1^{a}	5.26^{c}	23.2^{b}	45.1^{a}	2.58	***	n.s	n.s
(E)-2-Nonenal†	1168	A	205^{d}	266^{bc}	354^{a}	212^{d}	251^{bcd}	296^{b}	152^{e}	227^{cd}	217^{cd}	10.7	***	***	**
(E)-2-Decenal	1266	A	151de	201^{c}	253^{b}	306^{a}	171^{cd}	150de	111^{ef}	142de	85.8^{f}	12.0	n.s	***	***
(E,E)-2,4-Decadienal†	1333	A	379^{cd}	353^{d}	399^{cd}	1960^{a}	713^{b}	374^{d}	574^{bc}	493^{cd}	399^{cd}	84.2	***	***	***
n-9 lipid derived															
Octanal†	1007	A	325^{d}	550^{b}	796^{a}	398^{cd}	458^{bc}	469^{bc}	330^{d}	476^{bc}	379^{cd}	25.6	***	***	***
1-Octanol	1073	A	82.2^{c}	319^{b}	599^{a}	45.8^{cd}	81.1^{c}	84.2^{c}	26.9^{d}	84.2^{c}	41.6^{cd}	30.5	***	***	***
Nonanal†	1107	A	2640^{bc}	3860^{a}	3720^{ab}	2470^{c}	2610^{bc}	3100^{abc}	2150^{c}	2420^{c}	2470^{c}	148	n.s	*	n.s
1-Nonanol	1173	A	72.1^{c}	370^{b}	685^{a}	n.d.^{c}	n.d.^{c}	n.d.^{c}	5.71^{c}	n.d.^{c}	n.d.^{c}	40.0	***	***	***
Decanal†	1209	A	346de	763^{b}	941^{a}	278^{e}	538^{c}	780^{b}	258^{e}	403^{d}	425^{cd}	41.2	***	***	**
Ketones															
2-Heptanone	891	A	189^{ef}	269de	360^{cd}	184^{ef}	414^{bc}	424^{bc}	136^{f}	532^{b}	790^{a}	35.2	***	***	***
2,3-Octanedione	988	A	124^{cd}	105^{d}	184^{abc}	135^{cd}	215^{ab}	150^{bcd}	114^{cd}	238^{a}	182^{abc}	10.6	*	n.s	*
2-Octanone	992	A	17.2^{f}	29.5^{de}	40.6^{cd}	15.2^{f}	36.7^{d}	54.4^{b}	19.0^{ef}	48.4^{bc}	101^{a}	4.40	***	***	***
2-Nonanone	1093	A	46.2^{cd}	126^{b}	192^{a}	30.6^{d}	46.6^{cd}	56.6^{cd}	30.7^{d}	48.6^{cd}	67.2^{c}	8.90	***	***	***
2-Decanone	1194	A	50.8^{d}	149^{b}	264^{a}	42.2^{d}	61.5^{d}	67.9^{cd}	41.9^{d}	68.1^{cd}	94.5^{c}	11.9	***	***	***
2-Undecanone	1293	B	35.8^{cd}	76.2^{b}	88.1^{a}	11.2^{g}	32.6^{de}	43.6^{c}	10.7^{g}	18.0^{fg}	24.0^{ef}	4.53	***	***	**
Long chain aldehydes															
Tetradecanal	1618	B	292^{c}	3300^{b}	4570^{a}	625^{c}	638^{c}	640^{c}	717^{c}	505^{c}	290^{c}	255	***	***	***
Pentadecanal	1715	B	2310^{c}	9230^{b}	11500^{a}	626^{cd}	962^{cd}	1050^{cd}	679^{cd}	708^{cd}	370^{d}	701	***	***	***
Hexadecanal	1818	B	47800^{b}	231000^{a}	233000^{a}	16700^{b}	31900^{b}	34300^{b}	19900^{b}	32200^{b}	23000^{b}	15100	***	***	***

CTG= Defatted meat sample containing chicken triglycerides; **CTGPL**= Defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w); **CPL**= Defatted meat sample containing chicken phospholipids; **SFTG**= Defatted meat sample containing sunflower triglycerides; **SFTGPL**= Defatted meat sample containing sunflower triglycerides and phospholipids (1:1 w/w); **SFPL**= Defatted meat sample containing sunflower phospholipids; **SYTG**= Defatted meat sample containing soy triglycerides; **SYTGPL**= Defatted meat sample containing soy triglycerides and phospholipids (1:1 w/w); **SYPL**= Defatted meat sample containing soy phospholipids; **C**= Class; **S**= Source; **CxS**= Class x source interactions

†Odour active compound

[1] Linear retention indices determined on DB-5 column (n.d. = not detected)

[2] Confirmation of identity where A = MS and LRI agree with those of authentic compounds; B = MS agrees with reference spectrum in NIST 14 MS database and LRI agrees with literature values in the Chemistry Web Book database

[3] Means of 4 replicates where the same letters within each row indicate no significant differences ($p = 0.05$)

[4]Significance of lipid class (C), source (S) and interaction of both (CxS) on the volatile compounds where *, ** and *** denote significant differences at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ respectively.

Table 4-4: Phospholipid classes in chicken, sunflower and soy phospholipids

Phospholipid class	Composition of phospholipid (%)		
	Chicken [a,b,c]	Sunflower [d,e,f]	Soy [e,f,g,h]
PC	46 – 61	31 – 45	22 – 35
PE	15 – 28	14 – 26	17 – 26
PI	6 – 11	14 – 32	16 – 18
PA	n.r.	2 – 6	6 – 11
SM	3 – 8	n.r.	n.r.

PC= Phosphotidycholine; PE= Phosphotidylethanolamine; PI= Phosphatidylinositol, PA= Phosphatidic acid; SM= Sphingomyelin; n.r= Not reported
Sources: [a]Marion and Miller (1968) [b]Peng and Dugan Jr. (1965) [c](Ferioli & Caboni, 2010) [d]Chapman (1980) [e]Lončarević et al. (2016) [f]Doert et al. (2012) [g]van Nieuwenhuyzen and Tomás (2008) [h]Balazs et al. (1996)

4.6.2 Figures

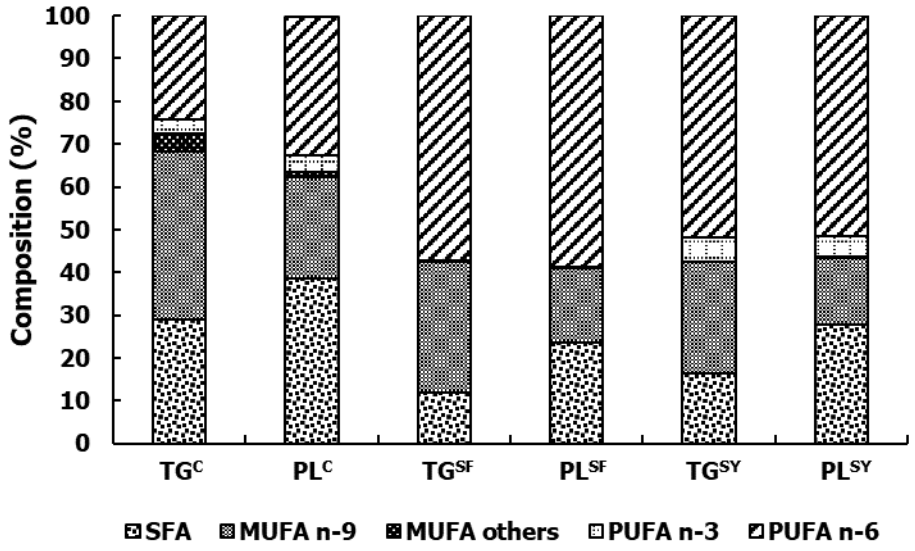


Figure 4-1: Fatty acid class composition (%) of triglyceride (TG) and phospholipid (PL) from chicken (^{C}), sunflower (^{SF}) and soy (^{SY})

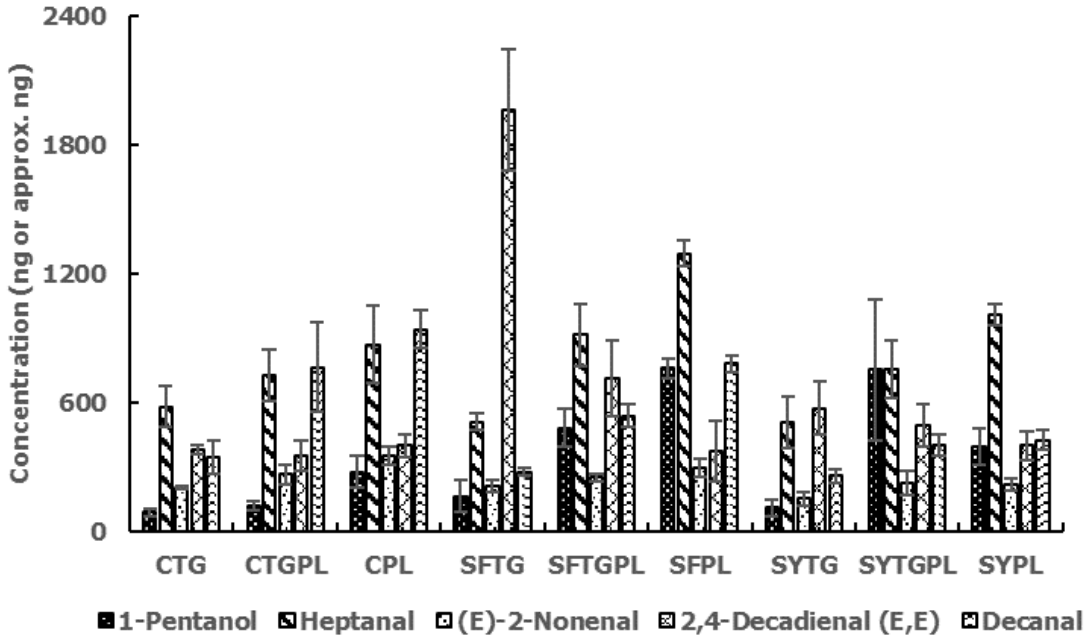


Figure 4-2: Mean quantities (approx. ng) of selected lipid-derived volatiles in LN aroma extracts of reconstituted samples (C/SF/SYTG= defatted meat sample containing chicken/ sunflower/ soy triglycerides; C/SF/SYTGPL= defatted meat sample containing chickens/ sunflower/ soy triglycerides and phospholipids (1:1 w/w); C/SF/SYPL= defatted meat sample containing chicken/ sunflower/ soy phospholipids; n=4 with error bars representing standard deviation of replicates)

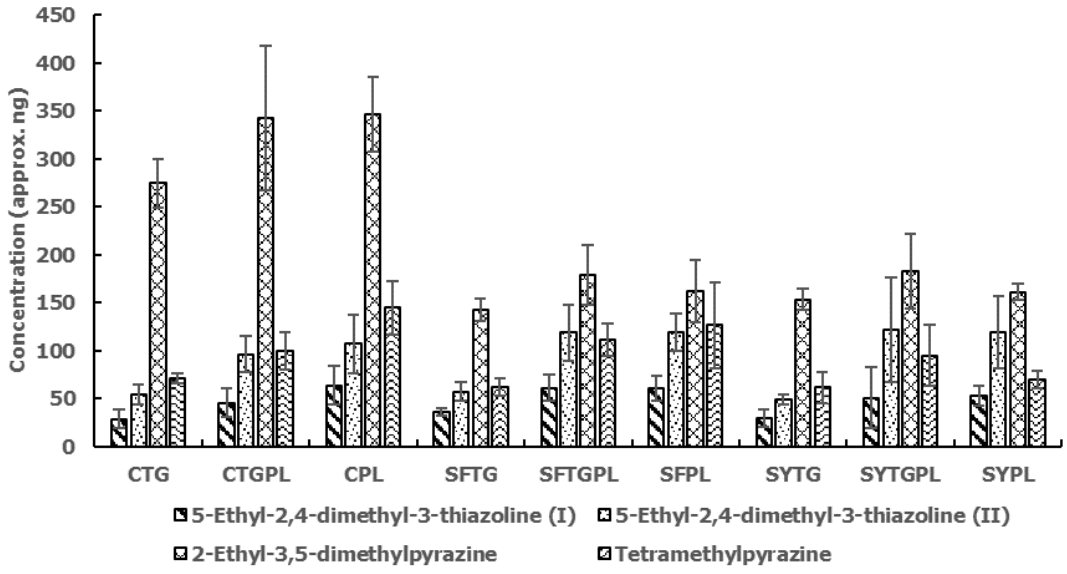


Figure 4-3: Mean quantities (approx. ng) of thiazolines and pyrazines in LN aroma extracts of reconstituted samples (C/SF/SYTG= defatted meat sample containing chicken/ sunflower/soy triglycerides; C/SF/SYTGPL= defatted meat sample containing chickens/ sunflower/ soy triglycerides and phospholipids (1:1 w/w); C/SF/SYPL=

defatted meat sample containing chicken/ sunflower/ soy phospholipids; n=4 with error bars representing standard deviation of replicates)

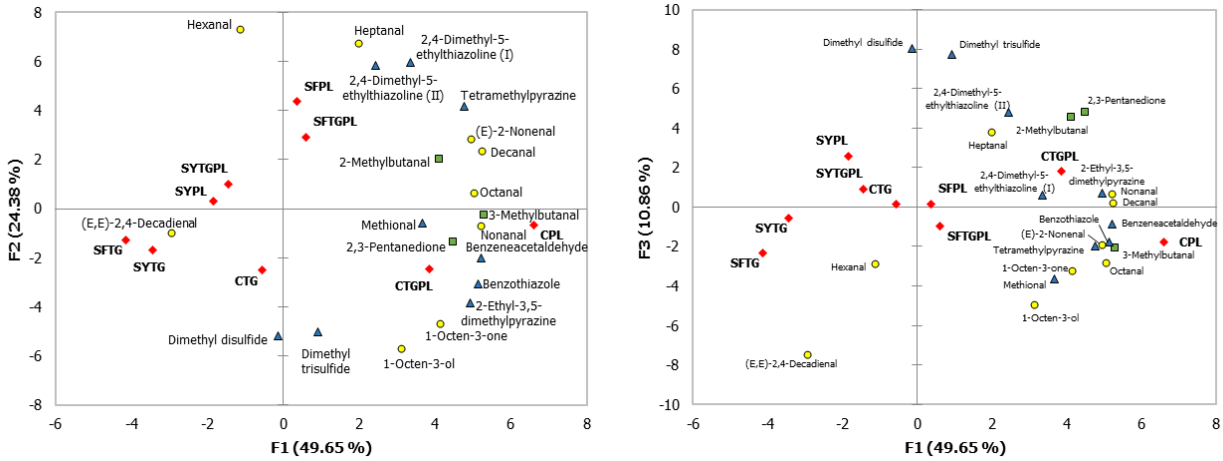
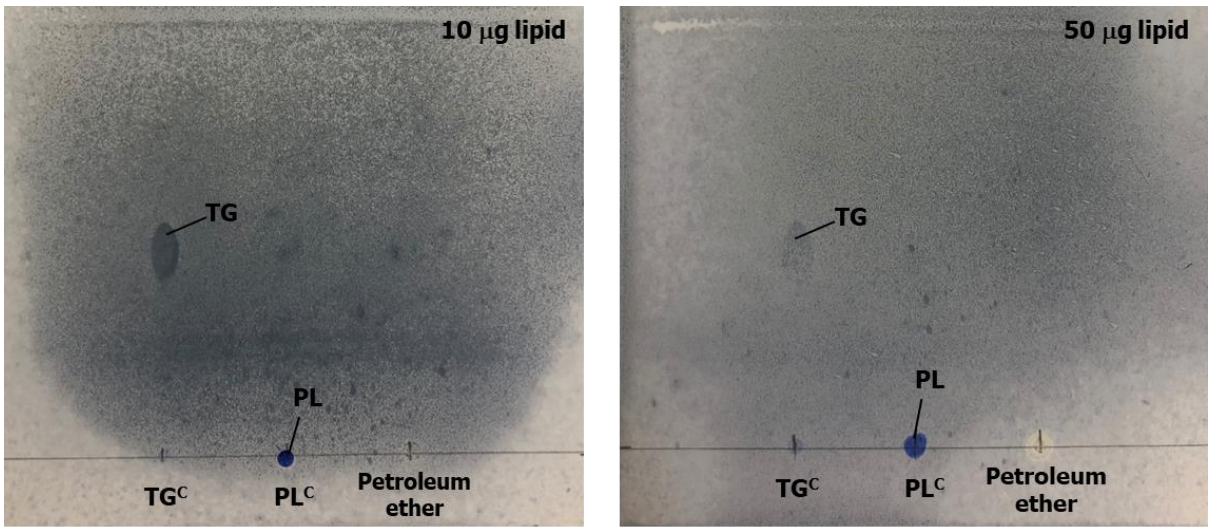


Figure 4-4: Principal component analysis of reconstituted samples (C/SF/SYTG= defatted meat sample containing chicken/ sunflower/ soy triglycerides; C/SF/SYTGPL= defatted meat sample containing chickens/ sunflower/ soy triglycerides and phospholipids (1:1 w/w); C/SF/SYPL= defatted meat sample containing chicken/ sunflower/ soy phospholipids; n=4) showing correlations with odour-active volatile compounds (□: Maillard reaction product; ∆: S-/N-/O-containing compound; •: lipid degradation product)

4.7 Appendices

Appendix G: Thin layer chromatography of purified chicken triglyceride (TG^C) and chicken phospholipid (PL^C) eluted with hexane: diethyl ether:formic acid (80:20:2 v/v)



Appendix H: Quantification ions and relative response factors used in the semi-quantification of compounds

Compound	LRI	Quant ion	RRF
3-Methylbutanal	657	58	7.50
2-Methylbutanal	664	58	8.35
2,3-Pentanedione	696	100	23.9
Dimethyl disulfide	746	94	3.27
1-Pentanol	778	70	11.2
Hexanal	802	82	33.1
(E)-2-Hexenal	864	69	9.84
2-Heptanone	891	43	2.57
Heptanal	903	70	11.2
3-(Methylthio)propanal	912	48	4.88
(E)-2-Heptenal	963	68	28.6
2-Isopropylpyrazine (IS)	974	107	3.55
1-Octen-3-one	978	70	4.77
1-Octen-3-ol	983	72	15.1
Dimethyl trisulfide	984	126	3.98
2,3-Octanedione	988	71	8.74
2-Pentylfuran	992	138	12.8
2-Octanone	992	128	37.2
(E)-2-(2-Pentenyl)furan	1001	136	11.0
Octanal	1007	84	18.0
2-Phenylacetaldehyde	1058	91	2.05
(E)-2-Octenal	1063	83	18.9
(E)-2-Octen-1-ol	1071	55.	17.0
1-Octanol	1073	84	15.8
4-Nonanone (IS)	1074	99	15.4

5-Ethyl-2,4-dimethyl-3-thiazoline (I)	1080	143	16.9
2-Ethyl-3,5-dimethylpyrazine	1084	135	2.73
Tetramethylpyrazine	1091	54	3.41
2-Nonanone	1093	58	4.51
5-Ethyl-2,4-dimethyl-3-thiazoline (II)	1099	143	18.4
Nonanal	1107	57	9.34
(E)-3-Nonen-2-one	1144	125	9.80
(E)-2-Nonenal	1168	70	12.1
1-Nonanol	1173	83	24.0
2-Decanone	1194	58	3.33
Decanal	1209	55	16.8
Benzothiazole	1235	135	2.94
(E)-2-Decenal	1266	70	14.0
2-Undecanone	1293	58	3.55
(E,E)-2,4-Decadienal	1333	81	3.86
Tetradecanal	1618	57	14.0
Pentadecanal	1715	57	11.7
Hexadecanal	1818	57	14.7

LRI= Linear retention indices determined on DB-5 column; Quant ion= Quantification ion; RRF= Relative response factor; IS= Internal standard

5. General discussion, conclusions and future work

5.1 General discussion and conclusions

This work started with a comparison of the key aroma volatiles in boiled beef (BB) and boiled chicken (BC) based on LN-SDE, followed by GC-O and GC-MS analysis in Chapter 2. To the best of my awareness, the latest study which involved a comparison between BB and BC aroma using the same cooking and extraction technique was by Gasser and Grosch (1990). Thus, it would be useful to update our knowledge of the key volatiles present in these meat aromas.

LN-SDE was selected as the aroma extraction technique as it provides the advantage of in-situ cooking and extraction, which facilitates the mimicking of meat cooking in stocks and stews in a realistic kitchen setting and avoids the loss of aroma during sample transfer inevitable in other methods. However, it is acknowledged that a frequently cited drawback of LN-SDE is artifact production due to contamination, oxidation or thermal reactions (Chaintreau, 2001). While it is possible that the occurrence of further reactions during the concentration of the aroma extract could lead to the formation of new compounds not derived from the cooking process, it is worthwhile to note that such a concentration step is also common in a kitchen setting where stews and bouillons are simmered down to obtain a more intense flavour, albeit in a different medium (water v.s. solvent). Nonetheless, caution can be practised by adding the precursors of the suspected artifact in the extract solvent and performing the concentration under the same conditions to investigate the likelihood of the formation of the target compound during this step. However, a possibility of the formation of the compound during concentration does not negate its possibility of formation during the cooking process.

Taken all the above into consideration, it can be difficult to pinpoint what constitutes an artifact.

In agreement with literature, it was shown in this experiment that the major classes of volatiles in both BB and BC extracts comprised sulfur- and/or nitrogen-containing compounds and short-chain branched aldehydes (e.g. Strecker aldehydes), which were mainly derived from the Maillard reaction, as well as straight-chain saturated and unsaturated aldehydes, alcohols and ketones, which originated from lipid degradation. In addition to this well-established knowledge, it was also discovered that 3-thiazolines could be important contributors to boiled meat aroma due to their high odour intensity scores and meat-like odours. The majority of the volatiles were present in both extracts. Notably, bis(2-methyl-3-furyl)disulfide (beef fat, meaty, fatty) was only found in the BB extract and it was possible that this compound was a key volatile in BB but not BC, which was also reflected in the higher flavour dilution (FD) factor of this compound reported in BB (2048) as compared to BC (<16) by Gasser and Grosch (1990). Although these researchers also found that the FD factor of (E,E)-2,4-decadienal in BC (2048) was much higher than BB (64), it could not be concluded in this work that this compound is a key volatile in BC based on mere differences in odour intensities (9 in BC; 5 in BB out of a total possible odour intensity score of 60). In order to confirm hypothesis #1 (differences between BC and BB aroma profiles lie in the presence of different ratios of key volatiles), quantification of the BC and BB extracts using internal standards could be performed. Nevertheless, the identification of the key volatiles in BC and BB was achieved in this chapter, which laid the groundwork for the subsequent experiments.

In Chapter 3, the role of chicken triglycerides (TG^C) and phospholipids (PL^C) in BC aroma generation was investigated using a defatted chicken meat matrix based on the key aroma volatiles identified in the previous chapter, in addition to other common but often less odour-

active Maillard reaction and lipid-derived products. The use of a defatted chicken meat matrix, albeit a closer system to real meat as compared to model reactions, presented limitations in that each processing step taken was a deviation from authenticity. The mincing, freeze-drying and lipid extraction steps would have resulted in a severe disruption in the integrity of the cell structure as previously reported in freeze-dried minced beef (Aksoy et al., 2019). This could have been evidenced using cryo-scanning electron microscopy (SEM), which had been employed in studies investigating the effect of freezing (Egelandsdal et al., 2019) and cooking (García-Segovia et al., 2007) on meat cell structure. Nevertheless, it was still a useful matrix for the controlled study of the effect of different classes of lipids on meat aroma generation.

In the kinetics experiment, labelled (E,E)-2,4-decadienal was added as an internal standard prior to HS-SPME-GC-MS analysis for the quantification of (E,E)-2,4-decadienal to avoid the degradation of the labile compound during the heating process. Appropriate quantities of the internal standard could also be determined and spiked in the sample before heating to follow the thermal degradation pathways of (E,E)-2,4-decadienal and confirm some of the proposed explanations in the experiment. It was reported that the cleavage of the C=C bonds at C^2 and C^4 of (E,E)-2,4-decadienal will likely yield (E)-2-octenal and hexanal respectively (Matthews et al., 1971; Nawar, 1984; Zamora et al., 2015). Thus, the degradation of (E,E)-2,4-decadienal during heating could have been investigated further by monitoring the formation of its labelled or 4-deuterated breakdown products, i.e., hexanal-d4 and (E)-2-octenal-d4 (two deuterium atoms attached to the two last methyl group each).

This chapter concluded with the finding that PL^C were more efficient than TG^C in the generation of lipid-derived products, in part attributed to the higher concentration of polyunsaturated fatty acids (PUFAs) in PL^C and thus, validating hypothesis #2a (phospholipids are more reactive than triglycerides due to their higher concentration of unsaturated fatty acids) but

with the caveat that the unsaturated fatty acids are PUFAs and not monounsaturated fatty acids (MUFAs) since PL^{C} had a lower concentration of MUFAs than TG^{C}. However, fatty acid (FA) composition is not the only factor influencing the reactivity of PLs as their functional groups can confer distinct physicochemical properties, such as electrical charges (i.e. negative, neutral/ zwitterionic, positive), which in turn influence their interaction with the other system constituents, thereby altering the rate of lipid oxidation. Nevertheless, the catalytic role of PL^{C} in the reaction of the less reactive TG^{C} was demonstrated by the lack of differences in the abundance of lipid-derived volatiles even when half the quantity of PL^{C} in the sample was replaced by TG^{C}, thereby proving hypothesis #2b (phospholipids can catalyse the reactivity of triglycerides) to be true. In addition, the phospholipids also exerted some influence on the Maillard reaction, specifically the thiazolines and pyrazines, but not the other Maillard reaction products identified.

Finally, the use of sunflower triglycerides (TG^{SF}) and phospholipids (PL^{SF}), as well as soy triglycerides (TG^{SY}) and phospholipids (PL^{SY}), as a substitution for the chicken counterparts was explored in Chapter 4. While the chicken lipids were purified to prevent cross-contamination which might have occurred during extraction, the commercial sunflower and soybean oil (triglycerides) and lecithins (phospholipids) were used as provided. Although this would be the normal circumstance in a manufacturing plant or a kitchen setting, these lipids could have been purified for the purpose of investigation in this study. Commercial sunflower and soybean oils could have been stripped to remove minor polar components, such as tocopherols, free fatty acids, mono- and di-acylglycerols, phospholipids and hydroperoxides, by passing the oils through a silicic acid and activated charcoal chromatographic column using *n*-hexane as an eluent according to the procedure described by Waraho et al. (2009). The lecithins could have been purified using the same method as the chicken phospholipid. In addition, the antioxidant activity of the lipids could have been characterised by determining

their peroxide value, which measures the level of hydroperoxides formed via primary lipid oxidation (Gotoh & Wada, 2006). Other methods of measurement are discussed in the review by Shahidi and Zhong (2015).

Nevertheless, the objective of evaluating the use of plant lipids in chicken aroma generation was accomplished and it was proposed that PL^{SF} has potential for use in the substitution of chicken lipids in plant-based meat products. Unlike samples containing any of the TGs and those with $(TG^{SY})PL^{SY}$ which were more closely associated with only (E,E)-2,4-decadienal and hexanal, samples containing $(TG^{SF})PL^{SF}$ were more closely associated with a balanced mix of Maillard reaction and lipid oxidation products, which was similar to the samples containing $(TG^{C})PL^{C}$. Although both PL^{C} and PL^{SF} were high in unsaturated FA content, their FA compositions were vastly different in that there was a higher concentration of n-6 FAs but lower concentration of n-3 and n-9 FAs in PL^{SF}. In order to supplement hypothesis #3 (plant phospholipids could be used as a substitution for chicken phospholipids as they both have a high unsaturated fatty acid content), the phospholipid class composition could be investigated to understand some of the observations unexplained by FA composition.

In conclusion, this work had shown that:

1. Although bis(2-methyl-3-furyl)disulfide (beef fat, meaty, fatty) is a key volatile in BB aroma, the characteristic odours of BB and BC are likely to be a balanced contribution of each volatile present at different ratios.
2. PLs are more reactive than TGs due to their higher PUFA concentration.
3. PLs can play a catalytic role in the reactivity of TGs.
4. PL^{SF} can be a suitable alternative to PL^{C} despite the differences in FA composition, indicating that this is not the only factor to consider when substituting animal lipids with plant lipids.

These findings would be useful for food manufacturers who are keen on the creation and modification of process flavourings in products such as soups, stocks and bouillons, as well as those who are in search of animal lipid alternatives for application in plant-based meat products.

5.2 Future work

5.2.1 Quantification of odour-active volatiles in BB and BC extracts

In order to confirm hypothesis #1 (differences between BB and BC aroma profiles lie in the presence of different ratios of key volatiles), quantification of the volatiles in the extracts using internal standards could be performed to support the data available on the different odour intensities of the volatiles.

5.2.2 Characterisation of phospholipids

An understanding of the major subclass composition of the phospholipids (i.e. PC, PE, PS, PA, PI), as well as positional distribution of the fatty acids (i.e. *sn*-1 and *sn*-2), using techniques such as ^{31}P–NMR and HPLC–ELSD (Wang & Zhou, 2017), would be useful in rationalising the behaviour of the phospholipids, their interactions with the other chemical constituents in meat and as a consequence, their impact on meat aroma generation. It is apparent that knowledge of fatty acid composition could only partly clarify the trends of the aroma volatiles observed and detailed structural information could help to shed light on some of the unexplained observations. This would be useful in the selection of plant phospholipids with an ideal profile in terms of fatty acid and phospholipid class composition for the substitution of chicken phospholipids.

5.2.3 Sensory studies with trained and consumer panel

Although free choice profiling was employed in this study as a rapid sensory tool to investigate if differences could be perceived between samples with and without the addition of sugar and amino acid precursors which were lost during lipid extraction, further sensory evaluation could be carried out on samples containing different lipids. Quantitative descriptive analysis (QDA) could be performed with a trained panel to identify and quantify the odour and taste attributes of the samples. When reviewed with instrumental data, the sensory results would facilitate the understanding of the differences between the samples (containing plant lipids) and the target (containing animal lipid) and provide guidance on how the sensory profile of the samples could be moved towards the target (e.g. addition of antioxidants to reduce the quantities of lipid oxidation products if the samples containing plant lipids have a fattier profile).

5.2.4 Investigation using complex model systems

While the defatted meat matrix has proven to be useful in this study, it is nonetheless a complex system with many confounding variables. On the other end of the spectrum, model reactions using sugars and amino acids in buffer media are widely utilised due to its simplicity and the effect of lipids on meat aroma generation have been previously investigated (Farmer & Mottram, 1990; Farmer et al., 1989; Salter et al., 1988). To account for the heterogeneity of meat yet avoid the complexity of a complete matrix, future studies could explore the progressive integration of meat constituents, including but not limited to transition metal ions (e.g., Fe^{2+}), peptides or proteins (e.g., myosin, myoglobin), vitamins, minerals and enzymes, to model systems at a pH relevant to meat (usually pH 5.5 – 6). This would facilitate the deconvolution of the effects of interacting parameters and determination of the impact of phospholipids on meat aroma generation with less ambiguity. Complex systems could also include process reaction flavours involving raw materials such as yeast extracts, hydrolysed vegetable protein, enzyme digests, fermented extracts (e.g. soy), which are not usually

present in meat but added to the system to enhance the reaction and/or flavour, in addition to the common sources of sugars and amino acids. Within a chosen system, different quantities of the components could also be examined as extent of interactions and rate of reactions could be concentration dependent. A 1:1 w/w ratio of TG:PL was employed in this study for simplicity but concentrations closer to the natural abundance present in meat could also be investigated.

5.3 References

Aksoy, A., Karasu, S., Akcicek, A., & Kayacan, S. (2019). Effects of different drying methods on drying kinetics, microstructure, color, and the rehydration ratio of minced meat. *Foods, 8*(6), 216.

Chaintreau, A. (2001). Simultaneous distillation–extraction: from birth to maturity—review. *Flavour and Fragrance Journal, 16*(2), 136-148.

Egelandsdal, B., Abie, S. M., Bjarnadottir, S., et al. (2019). Detectability of the degree of freeze damage in meat depends on analytic-tool selection. *Meat Science, 152*, 8-19.

Farmer, L. J., & Mottram, D. S. (1990). Interaction of lipid in the maillard reaction between cysteine and ribose: The effect of a triglyceride and three phospholipids on the volatile products. *Journal of the Science of Food and Agriculture, 53*(4), 505-525.

Farmer, L. J., Mottram, D. S., & Whitfield, F. B. (1989). Volatile compounds produced in maillard reactions involving cysteine, ribose and phospholipid. *Journal of the Science of Food and Agriculture, 49*(3), 347-368.

García-Segovia, P., Andrés-Bello, A., & Martínez-Monzó, J. (2007). Effect of cooking method on mechanical properties, color and structure of beef muscle (M. pectoralis). *Journal of Food Engineering, 80*(3), 813-821.

Gasser, U., & Grosch, W. (1990). Primary odorants of chicken broth. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 190*(1), 3-8.

Gotoh, N., & Wada, S. (2006). The importance of peroxide value in assessing food quality and food safety. *Journal of the American Oil Chemists' Society, 83*(5), 473-474.

Matthews, R. F., Scanlan, R. A., & Libbey, L. M. (1971). Autoxidation products of 2,4-decadienal. *Journal of the American Oil Chemists Society, 48*(11), 745-747.

Nawar, W. W. (1984). Chemical changes in lipids produced by thermal processing. *Journal of Chemical Education, 61*(4), 299.

Salter, L. J., Mottram, D. S., & Whitfield, F. B. (1988). Volatile compounds produced in maiilard reactions involving glycine, ribose and phospholipid. *Journal of the Science of Food and Agriculture, 46*(2), 227-242.

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods, 18*, 757-781.

Wang, T., & Zhou, D. (2017). Advances in phospholipid quantification methods. *Current Opinion in Food Science, 16*, 15-20.

Waraho, T., Cardenia, V., Rodríguez-Estrada, M. T., McClements, D. J., & Decker, E. A. (2009). Prooxidant mechanisms of free fatty acids in stripped soybean oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 57*(15), 7112-7117.

Zamora, R., Navarro, J. L., Aguilar, I., & Hidalgo, F. J. (2015). Lipid-derived aldehyde degradation under thermal conditions. *Food Chemistry, 174*, 89-96.