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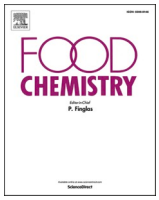

Effect of triglycerides and phospholipids on boiled chicken aroma generation

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ABSTRACT

Lipids, particularly polar phospholipids, are important precursors for the formation of species-specific meat aromas, but their role in the development of chicken aroma is not fully understood. 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 (mimicking the boiling and reduction of stock) and analysed 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 ($p < 0.05$). More importantly, PL^C could have an enhancement effect on the formation of key volatiles from TG^C. Small quantities of phospholipids could be used to promote the oxidation of triglycerides as a useful cost-saving strategy for manufacturers.

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 comprising either a glycerol (a trihydroxy sugar alcohol) or a sphingosine (a long chain amino alcohol) (Lehninger et al., 2012) and a phosphate moiety.

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. PLs were identified to be the greater culprit of meat rancidity as compared to TGs during the frozen storage of model systems made up of beef or chicken 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 TGs only enhanced the effects in the presence of PLs (Igene & Pearson, 1979).

Later, the research of Mottram and Edwards (1983) discovered the positive value of lipids, particularly PLs, in the formation of 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. On the other hand, the removal of both TGs and PLs resulted in the substitution of the meaty aroma by generic roasted notes, which was attributed to a 40-fold increase in the formation of dimethylpyrazines (isomers not specified), a 10-fold increase in methylpyrazine, and a decrease in aliphatic lipid-derived aldehydes. As pyrazines are typical products of the Maillard reaction, it was suggested that lipids or their degradation products acted as competitors during pyrazine formation.

Maillard-lipid interactions have since 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). Given the renewed interest in vegan alternatives, an elucidation of the role of PLs is worthy of investigation as there are still aspects of lipid oxidation, relative to TGs, and their interaction with the Maillard reaction, yet to be fully understood.

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The aim of this study is to investigate the role of TGs and PLs in the generation of boiled chicken (BC) aroma from both an aroma profile and time-dependence perspective. We used a defatted meat matrix with the addition of lipids to achieve a system as close as possible to real meat and mimic the cooking of stocks and stews in a kitchen setting by employing Likens-Nickerson simultaneous distillation-extraction (LN-SDE). TG^C, PL^C and a 1:1 *w*/w mixture of TG^C:PL^C, were added at a total concentration corresponding to the natural lipid levels found in chicken. These compositions were selected to facilitate a systematic comparison of the individual and combined effects of TG^C and PL^C on the generation of cooked chicken aroma compounds derived from both lipid and Maillard interactions. The hypothesis is that PLs are more reactive than TGs. This could be due to their higher proportion of unsaturated FAs or their emulsifying capacity, which facilitates the interaction of TGs with water soluble reactants.

2. Materials and methods

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) 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, Supelco 37 Component FAME Mix (CRM47885), amino acid standard (AAS18), and L-norvaline 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 (MCT, C8 – C10, ≥94%) were obtained from Chempri BV Oleochemicals (Raamsdonksveer, The Netherlands). High purity water (18.2 MΩ) was obtained from a Select Fusion Ultrapure water deionisation unit (SUEZ, Peterborough, UK) for instrumental analysis.

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.

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 (Fig. SF1 in Supplementary Information). As the purpose of the purification procedure was to obtain lipid fractions in sufficient yields for fatty acid characterisation and experiments, the achieved level of separation was deemed adequate for the study aim.

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 × 0.25 mm × 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 (%).

2.5. Likens-Nickerson simultaneous distillation-extraction experiment (LN-SDE)

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 1. To account for the effect of lipid content on flavour extraction, chemically unreactive MCT were added to the defatted matrix (DF) to standardise lipid content across systems. 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 ≥12 h equilibration. An internal standard mix (1 μL) of 2-isopropylpyrazine and 4-nonanone (both 10 ng μL^{-1}) was added to the sample the following day before extraction. A total of four replicates were prepared for each treatment.

Each 25 g sample was boiled at 100 °C in a heating mantle for 30 min

Table 1
Composition of reconstituted samples for LN-SDE experiment.

Component	Weight in reconstituted samples (g)				
	FD	DF	CTG	CTGPL	CPL
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.

to achieve a clear and flavourful stock then 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.

GC–MS analyses were performed on an Agilent 6890 N 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 × 0.25 mm × 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. Semi-quantification of the compounds was performed by comparison of their respective peak areas against that of the internal standard using single ions and relative response factors (RRFs) calculated for each compound from a clean spectrum as the ratio of the area of the TIC/area of the single ion. The internal standard for S- and/or N-heterocyclic compounds was 2-isopropylpyrazine (a similar molecular weight N-heterocycle which was not present in the samples) while 4-nonanone was used for the other compounds reflecting the longer hydrophobic carbon chains of the lipid-derived compounds. The quantification ions and RRFs used are listed in Table ST1 (supplementary information).

2.6. Headspace solid phase Micro extraction experiment (HS-SPME)

The composition of the reconstituted samples is listed in Table 2. All the samples were prepared in 20 mL glass vials, and the reconstituted samples were flushed under nitrogen for 5 min, lidded and sealed with parafilm, mixed using a magnetic stirrer until the lipid was incorporated and stored at 4 °C overnight for ≥12 h equilibration. Prior to heat treatment the following day, each vial was left open for 1 min to allow air to enter the headspace. The samples were heated in a water bath at 100 °C for a duration of 30, 60, 90, 120 and 150 min. An internal standard (5 μL) of 4-nonanone (100 ng μL^{-1}) was immediately added to the sample using a syringe injected through the PTFE/silicone-lined septum and placed on the instrument for SPME analysis without delay. A total of three replicates were prepared for each treatment.

Table 2
Composition of reconstituted samples for HS-SPME experiments.

Component	Weight in reconstituted samples (g)		
	CTG	CTGPL	CPL
Defatted meat	0.235	0.235	0.235
TG^C	0.020	0.010	–
PL^C	–	0.010	0.020
Water	0.745	0.745	0.745

TG^C = chicken triglycerides; PL^C = chicken phospholipids; 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.

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 7890 A 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 × 0.25 mm × 1 μm, Phenomenex, CA, USA). 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 (E,E)-2,4-decadienal (*m/z* 81, *m/z* 152) were performed. Compound identification and semi-quantification using 4-nonanone were as described in Section 2.5, to align with the LN-SDE experiment and since the majority of the lipid-derived volatiles were long chain carbonyl compounds. The quantification ions and RRFs used are listed in Table ST2 (Supplementary Information).

2.7. Statistical analysis

All statistical analyses were carried out using XLSTAT v2021.4.1 (Addinsoft Inc., Paris, France). Statistical differences between samples were tested using analysis of variance (ANOVA) and Fisher's least square significant difference (LSD) post-hoc test at a significance level of $p = 0.05$. Volatile data were analysed using principal components analysis (PCA) to provide a visual representation of the data.

3. Results and discussion

3.1. Fatty acid composition of lipids

The predominant FAs in TG^C and PL^C that were extracted from the meat samples 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 were mostly in close agreement with literature data (Ferioli & Caboni, 2010; Jahan & Paterson, 2007; Marion & Woodroof, 1965; Sahasrabudhe et al., 1985). Some variation 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).

PL^C had a higher concentration of SFAs and PUFAs than TG^C, and a lower concentration of MUFAs. C18:1n-9 accounted for the majority of the MUFAs present in both lipids but was present at a lower concentration in PL^C than TG^C (21.7% vs. 38.4%). The PUFA composition in TG^C and PL^C was predominantly made up of C18:2n-6 but there was more variation in that of PL^C with the presence of long chain FAs (≥C20) with ≥2C=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 higher proportion of n-9 FAs owing to the higher concentration of C18:1n-9, PL^C had a higher proportion 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

Table 3
Fatty acid composition of lipids (% of measured fatty acids, $n = 3$).

Fatty acid	TG^C	PL^C	p^1	Fatty acid	TG^C	PL^C	p^1
SFA				PUFA			
C6:0	0.04	0.15	***	C18:3n-3	3.07	1.69	**
C8:0	0.02	0.12	***	C20:3n-3	0.05	0.28	***
C10:0	0.03	0.10	***	C20:5n-3	0.07	1.02	***
C12:0	0.03	nd	***	C22:6n-3	0.06	0.83	***
C14:0	0.51	0.35	*	*t9,t12*-C18:2	0.03	nd	**
C15:0	0.09	0.13	***	*c9,c12*-C18:2	23.00	21.79	*
C16:0	22.1	26.2	**	C18:3n-6	0.19	0.10	ns
C17:0	0.13	0.19	***	C20:2n-6	0.24	1.13	***
C18:0	5.93	11.07	***	C20:3n-6	0.22	1.42	***
C20:0	0.08	0.04	ns	C20:4n-6	0.47	7.65	***
C21:0	0.02	0.24	*	C22:2n-6	nd	0.36	**
C22:0	0.03	nd	*	Total			
C24:0	0.02	0.12	ns	SFA	29.11	38.74	*
MUFA				MUFA	43.44	24.99	***
C16:1n-7	4.33	1.22	***	PUFA	27.38	36.27	***
C14:1n-9	0.13	nd	***	n-3	3.24	3.83	***
t9-C18:1	0.21	0.13	***	n-6	24.14	32.44	***
c9-C18:1	38.41	21.68	***	n-9	39.11	23.77	***
C20:1n-9	0.44	0.36	**				
C22:1n-9	0.03	nd	***				
C24:1n-9	0.01	1.60	***				

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; nd = not detected, [1]probability that there is a significant difference between means as determined by one-way ANOVA; ns = not significant; *, ** and *** denote significant differences at $p < 0.05$, $p < 0.001$ and $p < 0.0001$ respectively.

C20:4n-6 which were present in very low quantities in TG^C. Although the n-3 FAs were present within the same range 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 as compared to C18:3n-3, which was higher in TG^C compared to 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 of the cell membrane and performance of metabolic activities (Villaverde et al., 2006).

3.2. LN-SDE experiment

3.2.1. Effect of processing on volatile generation

The volatile compounds generated during the LN-SDE experiment are shown in Table 4. ANOVA showed that of the 41 volatiles analysed, 39 showed a significant difference across the different samples. The exceptions were dimethyl disulfide and dimethyl trisulfide which tend to have larger than average coefficients of variation. The abundance of volatiles generally increased with the extent of processing of the samples, i.e. FMM < FD < DF (with MCT) < CTG/CPL/CTGPL, 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 then come into contact more easily with the muscle lipids (Dominguez et al., 2019). 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 retention of volatile hydrophobic compounds in these dispersed lipid vesicles as compared to the original structured fat in the meat cells was also plausible. The subsequent freeze-drying process resulted in a change in the microstructure of minced meat. Aksoy et al. (2019) reported that in freeze-drying, whereby the drying temperature is below the glass transition temperature, to the formation of highly porous structures with enhanced surface area. Thus, 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 prior to any heat treatment would be more invasive than normal kitchen processing.

Although some compounds, such as 2-methyl-3-furanthiol, 2-furanmethanethiol and 2-mercapto-3-pentanone, were not found in these samples, it was likely that they were present in extremely low quantities owing to to the small sample size used but 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; Yeo et al., 2022) and the latter two both used 500 g of meat compared to the 25 g used in this experiment. 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 made it logistically challenging to accomplish. Although the processing steps inevitably caused some structural alterations to the matrix, the defatted meat system still retained characteristics of the native meat matrix and therefore provided a relevant system for studying flavour formation. A potential alternative for future consideration could be myofibrillar proteins as prepared in the work of Nishimura et al. (2010).

3.2.2. Effect of lipid class on volatile generation

3.2.2.1. Lipid-derived products. Significant differences in the abundance of lipid-derived volatiles were observed between the samples with 33 volatiles showing a significant difference between the three samples with added lipids. 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 Fig. 1 which illustrates some of the lipid-derived volatiles. This could be attributed to the significantly higher composition of n-3 FAs and n-6 FAs 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 (≥2C=C double bonds), such as C20:5n-3, C22:6n-3, C20:4n-6 and C22:2n-6 would lead to increased oxidative susceptibility and higher reactivity of PL^C (Dominguez et al., 2019).

The rate-limiting step in lipid oxidation 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).

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

Compound	LRI[1]	ID[2]	Mean quantities (approx. ng in sample) of key volatile compounds[3]						P[4]
			FMM[5]	FD	DF	CTG	CTGPL	CPL	
n-3 Lipid-derived									
(E)-2-Hexenal	864	A	nd	nd	9.7^{b}	13^{b}	33^{a}	17^{ab}	**
(E)-2-(2-pentenyl)furan	1001	A	nd	nd	15^{c}	9.9^{cd}	48^{a}	32^{b}	***
n-6 Lipid-derived									
1-Pentanol	778	A	87^{b}	10^{b}	99^{b}	89^{b}	277^{a}	118^{b}	***
Hexanal†	802	A	172^{b}	562^{b}	4790^{a}	3870^{a}	3570^{a}	4760^{a}	***
Heptanal†	903	A	69^{d}	192^{d}	514^{c}	581^{bc}	725^{ab}	870^{a}	***
(E)-2-Heptenal	963	A	70^{c}	3.6^{c}	243^{b}	271^{b}	410^{a}	313^{b}	***
1-Octen-3-one†	978	A	nd	nd	nd	34^{b}	40^{b}	50^{a}	***
1-Octen-3-ol†	983	A	63^{c}	141^{c}	659^{b}	709^{b}	993^{a}	776^{b}	***
2-Pentylfuran	992	A	26^{d}	76^{d}	48^{bc}	425^{c}	1110^{a}	733^{b}	***
(E)-2-Octenal	1063	A	15^{c}	94^{c}	718^{b}	679^{b}	1180^{a}	837^{b}	***
(E)-2-Octen-1-ol	1071	A	47^{c}	29^{c}	66^{c}	64^{c}	296^{a}	151^{b}	***
(E)-3-Nonen-2-one	1144	A	nd	nd	6.6^{cd}	9.9^{c}	38^{a}	25^{b}	***
(E)-2-Nonenal†	1168	A	nd	nd	220^{c}	205^{c}	266^{b}	354^{a}	***
(E)-2-Decenal	1266	A	68^{cd}	33^{d}	115^{bcd}	151^{bc}	253^{a}	201^{ab}	**
(E,E)-2,4-Decadienal†	1333	A	38^{c}	71^{c}	199^{b}	379^{a}	353^{a}	399^{a}	***
n-9 Lipid-derived									
Octanal†	1007	A	74^{d}	159^{d}	394^{c}	325^{c}	550^{b}	796^{a}	***
1-Octanol	1073	A	12^{d}	65^{cd}	48^{cd}	82^{c}	599^{a}	319^{b}	***
Nonanal†	1107	A	2410^{ab}	1200^{b}	2260^{ab}	2640^{ab}	3860^{a}	3720^{a}	*
1-Nonanol	1173	A	nd	nd	59^{c}	72^{c}	685^{a}	370^{b}	***
Decanal†	1209	A	85^{d}	72^{d}	682^{b}	346^{c}	763^{b}	941^{a}	***
Ketones									
2-Heptanone	891	A	3.6^{d}	26^{d}	161^{c}	189^{bc}	360^{a}	269^{b}	***
2,3-Octanedione	988	A	2.7^{d}	70^{c}	142^{ab}	124^{abc}	184^{a}	105^{bc}	***
2-Octanone	992	A	nd	nd	16^{c}	17^{c}	41^{a}	30^{b}	***
2-Nonanone	1093	A	nd	18^{de}	56^{c}	46^{cd}	192^{a}	126^{b}	***
2-Decanone	1194	A	2.8^{e}	13^{de}	43^{cd}	51^{c}	264^{a}	149^{b}	***
2-Undecanone	1293	B	4.3^{d}	16^{c}	27^{bc}	36^{b}	88^{a}	76^{a}	***
Long chain aldehydes									
Tetradecanal	1618	B	185^{c}	301^{c}	975^{c}	292^{c}	4570^{a}	3300^{b}	***
Pentadecanal	1715	B	273^{b}	1230^{b}	543^{b}	2300^{b}	11500^{a}	9230^{a}	***
Hexadecanal	1818	B	20800^{bc}	79000^{b}	13900^{c}	47,800	232000^{a}	231000^{a}	***
Maillard reaction products									
3-Methylbutanal†	657	A	18^{c}	30^{c}	30^{c}	54^{b}	54^{b}	74^{a}	***
2-Methylbutanal†	664	A	135^{b}	238^{a}	125^{b}	180^{ab}	219^{a}	183^{ab}	*
2,3-Pentanedione†	696	A	nd	154^{b}	155^{b}	426^{a}	436^{a}	484^{a}	***
Dimethyl disulfide†	746	A	795^{a}	729^{a}	623^{a}	774^{a}	1030^{a}	655^{a}	ns
3-(Methylthio)propanal†	912	A	206^{a}	275^{a}	88^{b}	252^{a}	240^{a}	272^{a}	**
Dimethyl trisulfide†	984	A	1440^{ab}	1310^{ab}	1030^{b}	1450^{ab}	2120^{a}	1430^{ab}	ns
2-Phenylacetaldehyde†	1058	A	1090^{c}	1220^{c}	1500^{bc}	2040^{ab}	2450^{a}	2660^{a}	***
5-Ethyl-2,4-dimethyl-3-thiazoline (I)	1080	A	87^{a}	38^{c}	35^{c}	28^{c}	45^{bc}	64^{ab}	***
2-Ethyl-3,5-dimethylpyrazine†	1084	A	60^{c}	139^{bc}	163^{b}	275^{a}	342^{a}	346^{a}	***
Tetramethylpyrazine†	1091	A	322^{a}	300^{a}	78^{c}	71^{c}	99^{c}	144^{b}	***
5-Ethyl-2,4-dimethyl-3-thiazoline (II)†	1099	A	111^{a}	59^{c}	68^{bc}	54^{c}	96^{ab}	107^{a}	**
1,3-Benzothiazole†	1235	A	nd	153^{c}	25^{d}	113^{c}	307^{b}	480^{a}	***

† 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 (https://webbook.nist.gov/).
[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 post-hoc test; nd = not detected.
[4] Probability that there is a significant difference between means as determined by one-way ANOVA where ns = no significant difference; *, ** and *** denote significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.
[5] 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.

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. Lipid oxidation likely proceeded in the order: CTG < CTGPL < CPL samples as TG^{C} and PL^{C} contained 3:2 and 2:3 MUFA:PUFA ratios, respectively.

The higher concentrations of C20:5n-3 and C22:6n-3 in PL^{C} as compared to TG^{C} could have compensated for the lower amount of C18:3n-3 in terms of oxidative reactivity. Thus, it was not surprising that n-3 derived lipid oxidation products such as (E)-2-hexenal 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) and C18:3n-3 (Ho et al., 1978). Meanwhile, the higher concentrations of C20:4n-6 in PL^{C} as compared to TG^{C} (7.6% vs. 0.5%) could have compensated for the lower amount of C18:2n-6 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 and (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 et al., 2000; Taylor & Mottram, 1990) and C18:2n-6 (Cossignani et al., 2014).

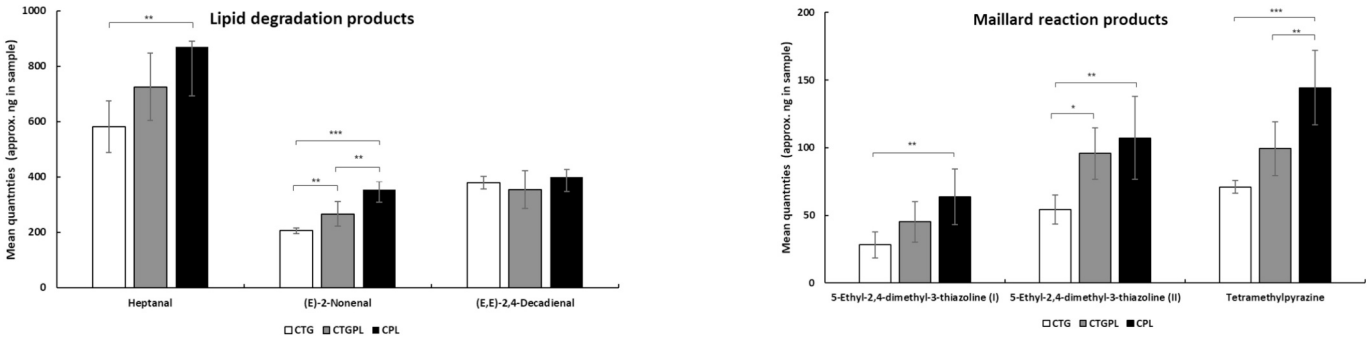

Fig. 1. Mean quantities (approx. ng) of selected lipid-derived volatiles and Maillard reaction products in LN aroma extracts of reconstituted samples □ = CTG (defatted meat sample containing chicken triglycerides); ▩ = CTGPL (defatted meat sample containing chicken triglycerides and phospholipids (1,1 w/w)); ■ = CPL (defatted meat sample containing chicken phospholipids); $n = 4$ with error bars representing standard deviation of replicates. Significant differences at $p < 0.05$, $p < 0.01$ and $p < 0.001$ are denoted by *, ** and *** respectively.

On the other hand, the lack of significance in hexanal and (E,E)-2,4-decadienal between CTG- and PL^C-containing samples could be due to the relative instability of these compounds during extended thermal processing. 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). Both of these could participate in condensation reactions, and (E)-2-octenal could form hexanal and acetaldehyde via retroaldol condensation. They could also be oxidised to their corresponding acids (i.e. hexanoic acid and decenoic acids).

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 compared to TG^C, there were more n-9 derived compounds, namely octanal, nonanal, decanal, 1-octanol and 1-nonanol, found in the samples containing CPL compared to CTG samples. This could be partly due to the differences in the physicochemical properties of TGs and PLs, which could influence the efficiency of lipid oxidation.

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 TGs with the other components in the system could have a limiting effect on its rate of participation in reactions (Farmer & Mottram, 1990), or it has been suggested that the relative thermal stability of TGs could retain aroma compounds (Nie et al., 2024), thereby inhibiting further reactions.

On the other hand, PLs offer excellent 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 PLs at the interfacial layer could produce an anionic surface charge 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). Not only were PLs, especially PE and PC, recognised as key lipids for the generation of aroma compounds (Chen et al., 2025; Liu et al., 2022; Liu et al., 2023; Nie et al., 2024), it was further postulated that they confer stability on the aroma compounds and contribute to their retention in the food matrix (Nie et al., 2024).

Relatively high concentrations of long chain aldehydes, especially hexadecanal, were detected upon addition of phospholipids. These aldehydes are released from plasmalogens upon hydrolysis (Fogerty et al., 1991; Salih et al., 1988). Plasmalogens are a class of phospholipids usually based on PE, which have a vinyl ether linkage in the *sn1* positions (replacing the more common ester linkage).

3.2.2.2. 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.

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 TGs and different classes of PLs 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 PL^C-containing samples. As seen in Fig. 1, 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 between the two. 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 been detected in boiled chicken and imparts a savoury;, grilled meat, fatty and juicy character. It can be formed from 3-mercapto-2-pentanone and acetaldehyde (Yeo et al., 2022). 3-Mercapto-2-pentanone is a Maillard reaction product which could be formed from the reaction between 4-hydroxy-5-methyl-3(2*H*)-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 thiamine 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 (E)-2-octenal and (E,E)-2,4-decadienal (Zamora et al., 2015). Since the acetaldehyde precursor could be influenced by lipid oxidation, it is 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 TGs as compared to PLs and a reduction in the formation of long chain heterocycles was also observed by Farmer and Mottram (1990) in model systems.

In comparison, for thiazoles, a closely related class of compounds, the addition of lecithin resulted in no marked changes in qualitative composition (Whitfield et al., 1988) and neither did the class of lipid have a significant effect on their 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 Fig. 1 is the presence of about twice the abundance of tetramethylpyrazine in CPL samples as compared to CTG samples, whereas there was no significant increase in 2-ethyl-3,5-dimethylpyrazine (Table 4). The formation pathways for substituted pyrazines are complex and matrix dependent. Subsequent to earlier reports that removal of PLs increased pyrazine formation (Mottram & Edwards, 1983); Whitfield et al. (1988) investigated 14 different pyrazines in a series of model systems and found that methylpyrazine decreased 3-fold when lecithin was added, however many pyrazines showed no change, and a few increased slightly. Interestingly 2-ethyl-3,6-dimethylpyrazine decreased upon addition of lecithin, but no change was observed for 2-ethyl-3,5-dimethylpyrazine in the cysteine/ribose model system. However, Farmer and Mottram (1990) showed in cysteine/ribose systems that pyrazines and thiazoles were largely unaffected by the addition of various PLs, but a large group of heterocyclic S-containing decreased in the presence of PLs. More recently, Yang et al. (2022) reported a decrease in 2,3-dimethylpyrazine when PLs were removed, but Chen et el., (2019) reported no change in tetramethylpyrazine when egg yolk PLs were added to chicken (in both instances these were the only pyrazines reported). Clearly, the effect of PLs on pyrazine formation is dependent on a number of factors which include the structure and source of the PLs, precursors, the matrix and time and temperature of heating.

The carbon skeleton of pyrazines is formed from dicarbonyls (glyoxal, methylglyoxal and 2,3-butanedione) or their respective hydroxycarbonyls, but formaldehyde and acetaldehyde can also provide methyl and ethyl substituents respectively (Kocadağlı et al., 2021; Low et al., 2007; Parker et al., 2010). Multiple formation pathways exist for most pyrazines, and the balance of pathways is determined largely by the availability of the precursors.

Although the dicarbonyls and hydroxycarbonyls are common reactive intermediates in the Maillard reaction, they are also formed during thermal treatment of fatty acids (Zhuang et al., 2022). From the results, we suggest that tetramethylpyrazine may rely more on lipid-derived precursors for its formation (demonstrating that lipid degradation products can participate in pyrazine formation), whereas the 2-methyl-3,5-diethylpyrazine is more dependent on reactive intermediates generated in the Maillard reaction. For tetramethylpyrazine, the rate limiting precursors may be 2,3-butanedione, whereas for 2-methyl-3,5-diethylpyrazine there are number of possible options and labelling studies are required to fully explain these differences. Looking specifically at how tetramethylpyrazine is influenced by the presence of PLs, neither Chen et al. (2019) nor Whitfield et al. (1988) found any significant change.

The data on odour-active volatiles is visually represented using a PCA plot as shown in Fig. 2. 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, with CPL at the positive end associated with most of the aroma compounds, while the second axis separated the Maillard reaction products from lipid-degradation products. 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 which promotes oxidation to a greater extent, as discussed at 3.2.1. In addition, the PL^{C}-containing samples were closely associated with a balanced mix of Maillard reaction and lipid oxidation.

3.3. Change in volatile profile over time (HS-SPME)

3.3.1. Effect of lipid class

This experiment focussed on just the lipid-derived volatiles which

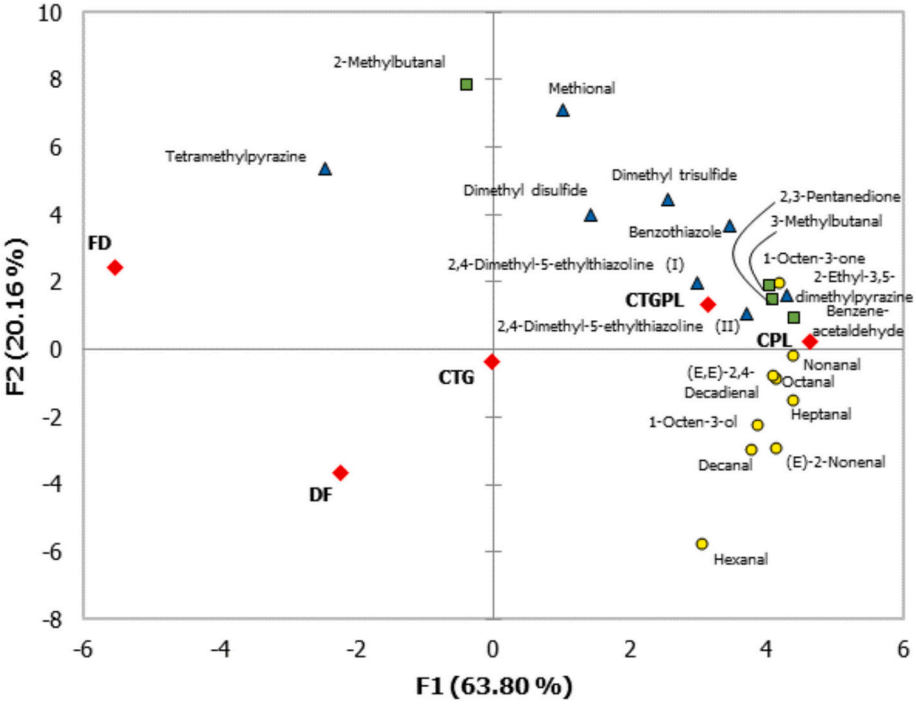

Fig. 2. Principal component analysis of reconstituted samples ◆ (FD = freeze-dried meat sample; DF = defatted meat sample containing medium chain triglyceride; CTG = defatted meat sample containing chicken triglycerides; CPL = defatted meat sample containing chicken phospholipids; CTGPL = defatted meat sample containing chicken triglycerides and phospholipids in 1:1 w/w) showing correlations with odour-active volatiles identified in LN aroma extracts. ■ Maillard reaction product; ▲ S-/N-/O-containing compound; ● lipid degradation product.

are shown in Figs. 3 and 4 and the full data are provided in Table ST3. Applying two-way ANOVA, all volatiles showed a significant difference with lipid class ($p < 0.001$), and with duration of heating ($p < 0.001$), except for (E,E)-2,4-decadienal which was not significant over heating duration. Interactions (heating duration x lipid class) were significant ($p < 0.05$) for the majority of the volatiles reflecting the fact that the trends over time were different in CTG, CTGPL and CPL samples.

The presence of phospholipids had a significant and substantial effect on the relative amount of lipid-derived compounds formed. They were found to be significantly higher in CTGPL and CPL samples compared to CTG samples. 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 the two PL^{C}-containing samples. Most of the volatiles (15 out of 21) were already significantly higher in CTGPL compared to CTG after 30 min heating duration and, after 150 min, all compounds were significantly higher in CTGPL.

However, there were very few significant differences between CTGPL and CPL samples. After 30 min, there were 10 compounds higher in CPL compared to CTGPL, but after 150 min, only 2-pentylfuran was significantly higher in CPL, and most compounds had decreased – 5 of them significantly ((E)-2-octenal, (E)-2-nonenal, (E,E)-2,4-decadienal, octanal and decanal).

The presence of PL^{C}, either alone or as a 1:1 *w*/w mix with TG^{C}, significantly increased lipid oxidation in the defatted and reconstituted chicken samples. This similarity between CTGPL and CPL samples 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 3.2.2, 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 PLs, 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).

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% vs. 27.4%) present in PL^{C} than in TG^{C}, especially the larger proportion of C20:4n-6 (7.65% vs. 0.47%), which would lead to increased oxidative susceptibility and higher reactivity as discussed in Section 3.2.2.

3.3.2. *Effect of heating duration*

The relative amount of lipid-derived compounds tended to increase as a function of heating duration, and this was evident in CTG samples (Figs. 3 and 4). On the other hand, in CTGPL and CPL samples, the increase did not continue indefinitely as precursors depleted and chemical reactions between lipid-derived volatiles and other intrinsic compounds within the meat matrix took place to form new secondary volatile and non-volatile compounds. For all compounds, when PL^{C} was present, accumulation slowed down leading to a plateau or a decrease with prolonged heating.

The overall trends over time for most of the volatiles were different for the 3 lipid compositions. In CTG samples, there was a steady increase in all lipid-derived volatiles over time, with higher amounts detected after each increment in heating duration. Using two-way ANOVA of the

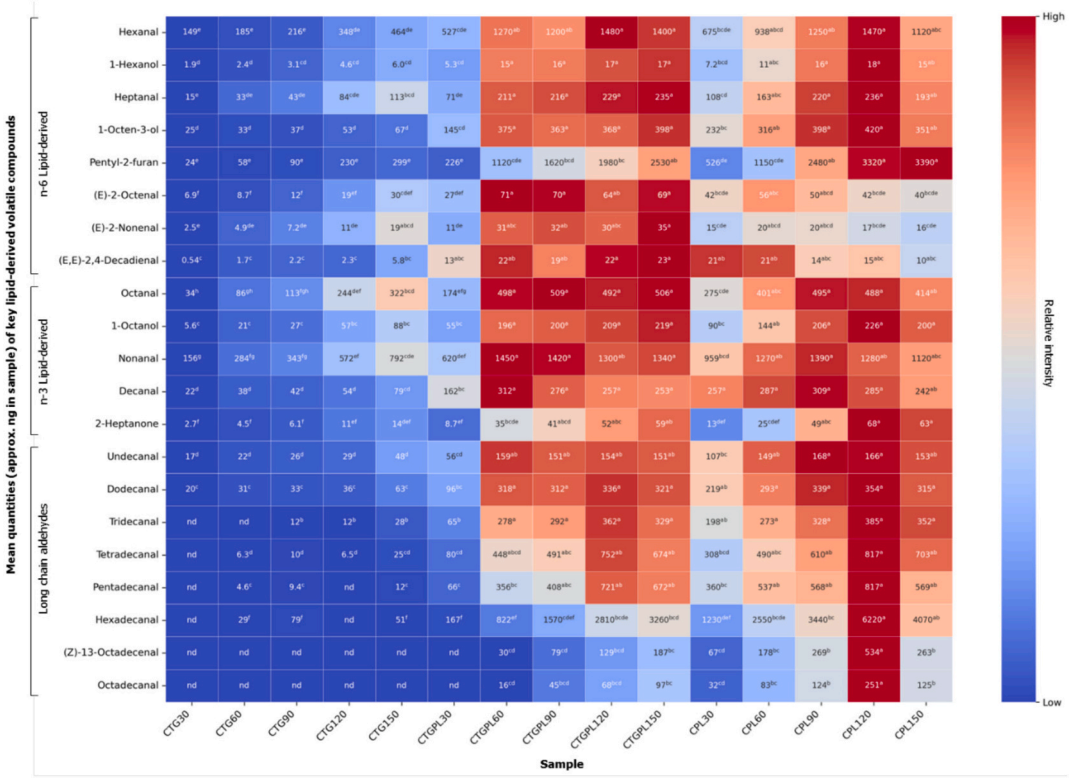

Fig. 3. Heatmap showing mean quantities (approx. ng in sample) of the key lipid-derived volatile compounds identified in the HS-SPME experiment (n = 3) over time, where CTG = defatted meat sample containing chicken triglycerides; CTGPL = defatted meat sample containing chicken triglycerides and phospholipids (1,1 w/w); CPL = defatted meat sample containing chicken phospholipids; numbers following sample names refer to heating duration (min). The same letters within each row indicate no significant differences (p = 0.05) using Fisher's LSD post-hoc test; nd = not detected.

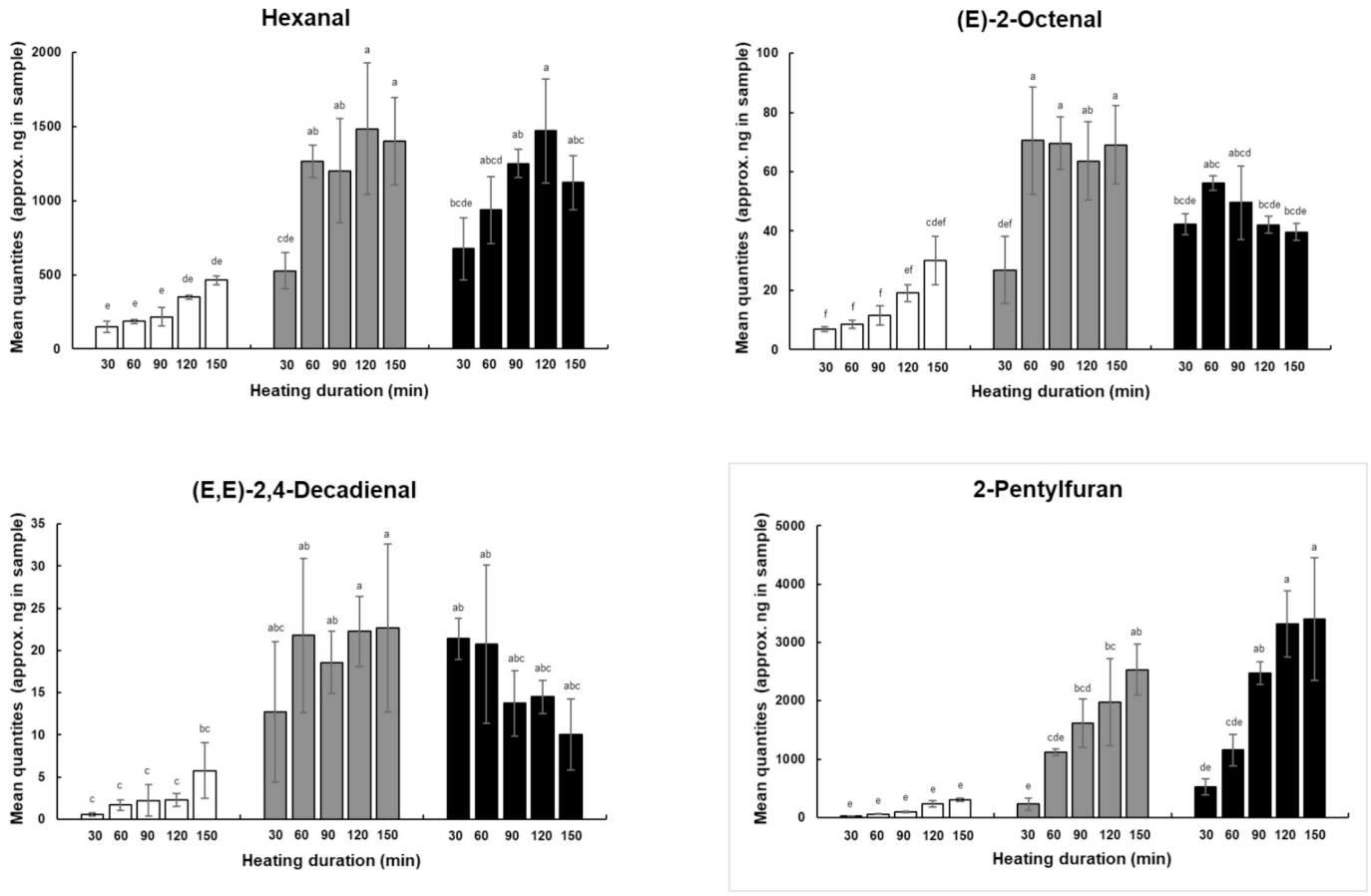

Fig. 4. Mean quantities (approx. ng in sample) of hexanal, (E)-2-octenal, (E,E)-2,4-decadienal and 2-pentylfuran in the headspace of reconstituted samples heated at 100 °C for different durations; □ = CTG (defatted meat sample containing chicken triglycerides); ▒ = CTGPL (defatted meat sample containing chicken triglycerides and phospholipids (1:1 w/w)); ■ = CPL (defatted meat sample containing chicken phospholipids); n = 3 with error bars representing standard deviation of replicates and same letters indicating no significant differences between samples (p = 0.05) as determined by Fisher's LSD post-hoc test.

full dataset, 9 volatiles were significantly higher after 150 min compared to 30 min. However, in a one-way ANOVA of just the CTG samples (effectively removing the higher standard deviation of the PL^C-containing samples), all the volatiles except two were significantly higher ($p < 0.05$) after 150 min compared to 30 min. In CTGPL samples, 20 out of 21 volatiles increased significantly between 30 min and 60 min when they tended to reach a plateau. There was no significant change during the final 30 min of heating. In CPL samples, not all volatiles increased significantly between 30 and 60 min and there was a tendency for compounds to decrease towards the end of the heating duration. This decrease was consistent, but not always significant, across the set of volatiles (see Table 5). In (E)-2-octenal, (E)-2-nonenal, nonanal and decanal compounds, heating to 150 min reduced the abundance to the extent that there was no significant difference between the samples after 30 and 150 min heating. The decrease was even more prominent for (E,E)-2,4-decadienal which was significantly lower at 150 min compared to 30 min.

The trends where a maximum is observed are typical for the formation of unstable compounds representing a balance between formation and degradation pathways. The formation of lipid degradation products increased as the content of PL^C increased, however there was a significant degradation pathway in the PL^C-containing samples, which was more obvious in the higher PL^C-containing sample. (E,E)-2,4-Decadienal 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 (accounting for both formation and degradation) is necessary. In CTG samples, the amount of (E,E)-2,4-decadienal increased incrementally with increasing heating duration from 0.40 ng after 30 min to 4.3 ng after 150 min. In CTGPL samples, quantities increased from 9.5 to 17 ng over the same period. However, the opposite trend was observed in CPL samples where (E,E)-2,4-decadienal was generated in significant amounts after 30 min (16 ng) but its abundance significantly decreased upon further heating to 7.5 ng after 150 min. 2,4-Alkadienals and 2-alkenals are highly reactive and unstable compounds (Zamora et al., 2015) which are susceptible to further degradation via autooxidation, photooxidation, condensation reactions 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), and (E)-2-octenal can form yet more hexanal in a retroaldol condensation. Whilst hexanal increased in abundance during heating but it is not possible to infer whether or not the degradation of (E,E)-2,4-decadienal contributed to this. Another possible explanation for the significant drop in (E,E)-2,4-decadienal is the reaction with the ε-amino groups of lysine incorporated in the protein (Alaiz & Barragán, 1995).

However, this does not explain the fact that the degradation is only apparent in CTGPL and CPL samples. A better explanation is provided by Bacot et al. (2007), who observed that hydroxyalkenals formed adducts with the amino group of PE. This same reaction may occur with α,β-unsaturated aldehydes such as 2-octenal, 2-nonenal and 2,4-decadienal and perhaps to a lesser extent with saturated aldehydes. When comparing the first and last time points (i.e. 30 min vs. 150 min), there was usually an accumulation in the abundance of the saturated aldehydes, showing that any degradation pathways were minor compared to formation. For the more reactive 2-alkenals, this was true in CTGPL but

not CPL samples, where there was no significant difference between 30 min and 150 min. For the most reactive (E,E)-2,4-decadienal in CPL samples, there was a significant loss between 30 min and 150 min, suggesting that degradation was faster than formation. The greater decrease after extended heating in 2,4-alkadienals, compared to 2-alkenals, can be explained by the higher reactivity of 2,4-alkadienals. The greater decrease of 2,4-alkadienals in CPL compared to CTGPL samples can be explained by the greater concentration of PE in the PL system. This explanation is supported by the fact that 2-pentylfuran, also a lipid-derived compound but with no aldehyde group and therefore unable to react with PE, did not decrease significantly on extended heating and accumulated across all lipid classes.

4. Conclusion

Using a defatted chicken-based 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 TGs and PLs in the thermal generation of key volatiles in boiled chicken aroma. Lipid class was found to have a highly significant impact on the extent of the lipid oxidation, as well as exerting some influence on the Maillard reaction as observed in the higher quantity of some S- and/or N-containing compounds produced in systems containing PL^C as compared to CTG. Besides demonstrating the higher efficiency of PL^C in generating lipid-derived volatiles, by following the change in profile over time, we demonstrated the enhancement effect of PLs in the lipid oxidation of the less reactive TGs. However, with prolonged heating in the presence of phospholipids, the more reactive unsaturated aldehydes showed a tendency to degrade. The knowledge that small quantities of PLs could be used to promote the oxidation of TGs at an adequate rate to maximise the thermal generation of key volatiles from TGs would be a cost-effective solution. In conclusion, this study has confirmed the hypothesis that PLs are more reactive than TGs. This could be attributed to the greater abundance and diversity of long chain PUFAs with $\geq$2C=C double bonds in PLs, which provided highly reactive precursors to initiate lipid oxidation. The emulsifying properties of PLs merit further investigation to understand how their amphiphilic nature could optimise reaction interfaces and enhance reactivity. Future studies could also incorporate comprehensive lipid profiling of TGs and PLs, beyond FA composition, to elucidate the contributions of molecular species and *sn* positional distribution to oxidative reactivity.

Although previous studies have shown phospholipids as an important contributor to meat flavour, they did not shed light on mechanisms. By systematically studying different meat-like model systems, and following the changes over time, this study provides further insight into the mechanisms. We show that some Maillard reaction products may be formed from lipid-derived precursors and demonstrate that excessive thermal processing can reduce some of the desirable volatiles. This study demonstrates why it is so critical to understand the role of different classes of lipids and their interaction with the Maillard reaction to fully control formation of meaty aromas, particularly in plant-based meat analogues.

CRediT authorship contribution statement

HuiQi Yeo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Dimitris P. Balagiannis:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Conceptualization. **Melissa Lim:** Writing – review & editing, Formal analysis, Data curation. **Jean H. Koek:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization. **Jane K. Parker:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.149082.

Data availability

Data will be made available on request.

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