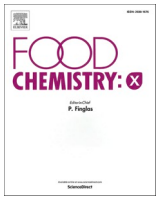
Food Chemistry: X 20 (2023) 100945

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



The effect of lutein on the oxidation of egg yolk phospholipids in a liposome model

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ARTICLE INFO

Keywords:
Phospholipid
Lutein
Liposomes
Antioxidant
^{1}H NMR

ABSTRACT

Egg yolk phospholipids (PLs) extracted by organic solvent are prone to oxidation, while they are quite stable in egg yolk. This study was to verify the decisive role of lutein (naturally present in egg yolk) on the oxidative stability of PLs by constructing liposome. The liposome samples were heated at 100 °C for 10 min, and then the oxidation products of the liposome samples were analyzed by ^{1}H NMR and GC–MS. The results showed that the concentrations of most of the oxidation products in the PLs-liposomes sample were significantly higher than those in the PLs&lutein-liposomes sample. Therefore, lutein could protect egg yolk PLs from oxidation, thus inhibiting the formation of lipid-derived volatile compounds. As those lipid-derived volatile compounds are the key fatty note odorants, this study has confirmed that the removal of lutein is the determining factor in using egg yolk PLs as an ideal precursor of fatty note odorants.

1. Introduction

Egg yolks are rich in lipids, especially phospholipids (PLs) (Xiao et al., 2020). Egg yolk PLs are prone to oxidation in comparison with neutral lipids (Chen, Balagiannis, & Parker, 2019a). PLs are the critical precursors of meat distinct aroma (Mottram, 1998). In a recent review (Chen, Wan, Yao, Yang, & Liu, 2023), egg yolk PLs can produce large quantities of 2,4-decadienals, which are the key fatty note odorants for chicken meat and fried foods. For this reason, egg yolk PLs have been successfully applied to enhance the aroma of chicken meat, fish and chips (Ye et al., 2022; Guo et al., 2021; Chen et al., 2019a). Meanwhile, the aroma of chicken meat was successfully mimicked with lutein-free egg yolks (Lutein, which is naturally present in egg yolk, was removed by solvent. Thus, lutein-free egg yolks were obtained.) when being heated to 100 °C for 10 min; but in the presence of lutein or antioxidant (*tert*-butylhydroquinone), it was egg yolk aroma, rather than chicken meat aroma. Meanwhile, large quantities of 2,4-decadienals were detected in the heated lutein-free egg yolks, while only small quantities of 2,4-decadienals were detected in the control (Yang, Liu, Wan, Guo, & Chen, 2022). Therefore, the effects of lutein on the oxidation of egg yolk PLs need further study.

Lutein is the key fat-soluble pigment in egg yolk (Steiner, Mcclements, & Davidov-Pardo, 2018), and it could be co-extracted with lipids (Yang et al., 2022). Lutein is an oxygenated carotenoid, which contains 8 isoprene units as the basic structural backbone. Due to its specific highly unsaturated structure, lutein is susceptible to oxidative degradation by oxygen and heat (Aparicio-Ruiz, Mínguez-Mosquera, & Gandul-Rojas, 2011); lutein also serves as an antioxidant through eliminating free radicals, quenching singlet oxygen and blocking lipid peroxidation chain reactions (Manzoor, Rashid, Panda, Sharma, & Azhar, 2022; Cantrell, Mcgarvey, Truscott, Rancan, & Böhm, 2003). Liposomes are widely used to encapsulate fat-soluble lutein, because of the higher encapsulation rate, uniform particle dispersion, with amphiphilic substances and more significant antioxidant effects (Steiner et al., 2018). PLs are ideal materials for liposome preparation, and ethanol injection method is widely used to prepare liposomes (Liu,

Abbreviations: DHA, docosahexaenoic acyl groups; EDTA, ethylenediaminetetraacetic acid; EPA+ARA, Eicosapentaenoic+Arachidonic acyl groups; GC–MS, gas chromatography-mass spectrometry; HS-SPME, Headspace solid-phase microextraction; Ln, Linolenic acyl groups; L or DU, linoleic or diunsaturated omega-6acyl groups; MDA, malonaldehyde; NMR, nuclear magnetic resonance; O or MU, oleic or monounsaturated acyl groups; OPLS-DA, orthogonal partial least squares-discriminant; PDI, polydispersity index; PLs, phospholipids; POV, peroxide values; S + M, saturated plus modified acyl groups; TBA, thiobarbituric acid; TMS, tetramethylsilane; VIP, variable importance in projection; ω-3, total omega-3 acyl groups.

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https://doi.org/10.1016/j.fochx.2023.100945
Received 25 June 2023; Received in revised form 22 September 2023; Accepted 13 October 2023
Available online 15 October 2023

Wang, Zou, Luo, & Tamer, 2020).

The oxidation of lipids has been comprehensively reviewed, and the oxidation products are also well summarized (Choe & Min, 2006; Frankel, 1991; Yin, Xu, & Porter, 2011). Analytical methods, including ^{1}H NMR and GC–MS, as well as in combination with chemical measurements are used to investigate the oxidation of lipids (Chen et al., 2023; Martin-Rubio, Sopelana, & Guillen, 2019). ^{1}H NMR is a powerful technique for differentiating acyl groups and oxidation products in lipids (Martínez Yusta, Goicoechea, & Guillén, 2014; Martin-Rubio, Sopelana, Ibargoitia, & Guillén, 2018; Vidal et al., 2023; Chen et al., 2022). In our previous reports, ^{1}H NMR has been applied to monitor the evolution of egg yolk PLs at 160 ℃ (Zhao et al., 2023), and the results demonstrated that egg yolk PLs were prone to oxidation and they were an ideal flavor precursor for fried foods (Chen, Balagiannis, & Parker, 2019b).

Although the antioxidant properties of lutein have been extensively studied (Haila, Lievonen, & Heinonen, 1996), as far as we are aware, little research about lutein protecting PLs from oxidation has been published. The purpose of this research was to investigate the effects of lutein on the oxidation of egg yolk PLs by comparing the oxidation products of PLs-liposomes and PLs&lutein-liposomes using ^{1}H NMR and GC–MS, thus providing insights for the effect of lutein on using egg yolk PLs as an ideal precursor of fatty note odorants.

2. Materials and methods

2.1. Reagents and chemicals

Lutein (purity > 95 %) was obtained from Solarbio Co. Ltd. (Beijing, China). Deuterated chloroform ($CDCl_3$) and 1,2-Dichlorobenzene were purchased from McLean Co. Ltd. (Shanghai, China). Aroma chemicals used as references: hexanal, methional, hexanal, methional, (E)-2-decenal, (E)-2-undecenal, (E)-2-octenal, undecanal, decanal, 2-pentylfuran, (E,E)-2,4-decadienal, (E)-2-nonenal, (E,E)-2,4-heptadienal, 1-octen-3-ol, benzaldehyde, (E,E)-2,4-nonadienal, and standards of *n*-alkanes (C5–C30) were from Sigma-Aldrich Co. Ltd. (Shanghai, China).

High-purity egg yolk PLs (> 95 %) were used in our study, as described recently (Chen et al., 2019b). Lutein was co-extracted in the crude extraction, but it was removed when the crude extraction was dissolved in hexane and cold acetone was used to precipitate and wash PLs.

2.2. Preparation of PLs-liposomes

Lutein (2 mg) was dissolved in 10 mL ethanol with 2.00 g of egg yolk PLs at 50 °C. The solution of ethanol was rapidly injected into 40 mL of hydration medium (phosphate buffer, PBS, pH 7.4) using a syringe. Then it was hydrated for 30 min with a magnetic stirrer at 50 °C in a water bath. The residual ethanol in the suspension was removed by evaporation under reduced pressure at 50 ℃ for 30 min, and then PLs&lutein-liposomes were obtained (Liu, Wang, Zou, Luo, & Tamer, 2020). Similarly, PLs-liposomes were obtained by using the same method. The final sample was hermetically sealed in a vial and stored at 4 °C for a short time. PLs-liposomes and PLs&lutein-liposomes (6 mL) samples were sealed in 20-mL headspace vials, respectively, and then they were boiled at 100 ℃ for 10 min. Each experiment was done in triplicate.

2.3. Determination of encapsulation efficiency of liposomes

Lutein was dissolved in petroleum ether to prepare a 0.1 mg/mL solution. Then the solution was diluted with petroleum ether gradient to a concentration of 0–2.5 μg/mL. The standard curve of lutein was drawn with a UV spectrophotometer at $\lambda_{max} = 446$ nm by using petroleum ether as a blank sample.

PLs&lutein-liposomes (0.5 mL) were stirred well with petroleum ether (3 mL) for 3 min to dissolve them. After centrifugation for 5 min, the supernatant from the centrifuged samples was collected. This process was repeated twice. The supernatant was combined, and then was diluted to 10 mL. The absorbance value A was measured. Then the content of free lutein (C_{free}) was calculated by substituting it into the standard curve (Tan et al., 2013). The lutein encapsulation effciency (EE_{lutein}, %) was respectively computed using the following equations:

$$EE_{lutetin}(\%) = \frac{C_{total} - C_{free}}{C_{total}}$$

2.4. Zeta potential, particle size and polydispersity index analysis

One mL of liposomes was suspended in 10 mL of distilled water. Then 1 mL diluted liposomes were placed in a cuvette, and zeta potential, average particle size and polydispersity index (PDI) were measured by a zeta potential and laser particle size analyzer (Nano-ZS90X, Malvern Instruments Ltd., Malvern, UK).

2.5. Determination of peroxide value and malonaldehyde

The peroxide values (POV) were determined by the American Oil Chemists' Society official method 965.335 (AOCS, 2009). The concentration of malondialdehyde (MDA) was determined by TBA reaction spectrophotometry as described by Tan et al. (2013).

2.6. NMR spectra acquisition

Before the ^{1}H NMR experiments, the heated samples were freeze-dried. The method of Zhao et al. (2023) was adopted, with appropriate modifications (Supplementary Methods 1). ^{1}H NMR spectra were obtained by dissolving 100 mg of lyophilized sample in $CDCl_3$ with TMS as an internal standard. All samples were measured using an ultra-low temperature broadband liquid nitrogen probe NMR instrument (AVANCE III HD 500 spectrometer (Bruker, Karlsruhe, Germany).

2.7. HS-SPME-GC–MS analysis of volatile compounds

HS-SPME-GC–MS was used to analyze the volatiles by referencing and slightly adapting the method of Yang et al. (2022) (Supplementary Methods 2). The volatiles were extracted through continuously stirring (250 rpm) in a thermostat (60 °C, 40 min). The GC–MS system (7890B GC, equipped with 5977A MS, Agilent Technologies Co. Santa Clara, CA) and a DB-WAX capillary column (30 m × 0.25 mm × 0.25 μm; Agilent) were used. Compound identification followed the method described by Yang et al. (2022). The content of volatile compounds was estimated by comparing the peak area of the sample with that of 1,2-dichlorobenzene (130.6 ng/μL).

2.8. Statistical analysis

The experiment was conducted in triplicate and the mean ± standard deviation was calculated using the SPSS statistical package (SPSS 23, IBM) with one-way ANOVA and independent samples *t*-test. Graphics were drawn and processed using Origin 2018 and Adobe Illustrator 2020; OPLS-DA analysis was performed using SIMCA14.1 (Umetrics, Sweden).

3. Results and discussion

3.1. Properties of the liposomes

The effect of heating on the properties of the two liposome samples is shown in Supplementary Fig. 1. The average particle sizes of PLs-liposomes sample and PLs&lutein-liposomes sample were 77.39±1.67 nm and 94.91±2.25 nm, respectively. The particle sizes of these two liposomes were less than 100 nm (standard of nanoliposomes) (Tan et al., 2013), which could be regarded as nanoliposomes. Heat treatment

would cause the expansion of the liposomes, thus increasing the particle size of the liposomes. After 10 min of heat treatment, the size of the PLs-liposomes increased more than that of the PLs&lutein-liposomes, indicating lutein could inhibit the aggregation of the liposomes to some extent, which was in line with the findings of Tan et al. (2013), probably because lutein could inhibit the oxidation of the liposomes.

The PDI of unheated PLs-liposomes sample and PLs&lutein-liposomes sample were 0.23±0.01 and 0.23±0.03, respectively (Supplementary Fig. 1B). The narrow PDI indicates the presence of a homogeneous particle size distribution in the prepared liposomes. Moreover, heating led to an increase in PDI, indicating the particle size distribution became larger. However, there was no significant difference in PDI of the two liposomes under the same treatment.

Zeta potential is an essential indicator of the stability of liposomal dispersions and is related to the charge of the liposome in the dispersion. There was no significant difference between the absolute values of the potentials of the two liposomes (Supplementary Fig. 1C). Because heating could cause the expansion of the phospholipid layer, which led to an increase in particle size and decline in relative negative charge density (Huang et al., 2016), the absolute value of the zeta potential of liposomes decreased.

The encapsulation rate of PLs&lutein-liposomes prepared by the ethanol injection method was 88.64 % (Supplementary Fig. 1D), and it could be seen that the encapsulation efficiency of lutein showed a decreasing trend after heating because liposomes were of thermodynamically unstable system. The movement of phospholipid molecules was enhanced when heating, and physical destabilization phenomena such as aggregation, precipitation, and flocculation occurred quickly, resulting in the leakage of encapsulated substances in liposomes. In addition, the thermal oxidation of PLs resulted in the disruption of the membrane structure and led to a looser and more disordered permeability, which also reduced the encapsulation efficiency of lutein (An & Gui, 2017).

3.2. POV and MDA of the liposomes

Hydroperoxides are the primary oxidation products of lipids, and POV is a practical method to detect the oxidation of lipids (Choe & Min, 2006). The POVs of both samples (Supplementary Fig. 2A) increased significantly during the heating, but the POVs of PLs&lutein-liposomes sample were lower than those of the PLs-liposomes sample. Malondialdehyde (MDA) is a secondary oxidation product of lipids, which can reflect the final oxidation degree of liposomes (Choe & Min, 2006). Similarly, the results of MDA in both samples (Supplementary Fig. 2B) were in agreement with those of POVs. The results of POV and MDA indicated that both samples were subjected to oxidation during the heating, but the PLs&lutein-liposomes sample was more stable because of the protection of lutein. The antioxidant capacity of lutein could be

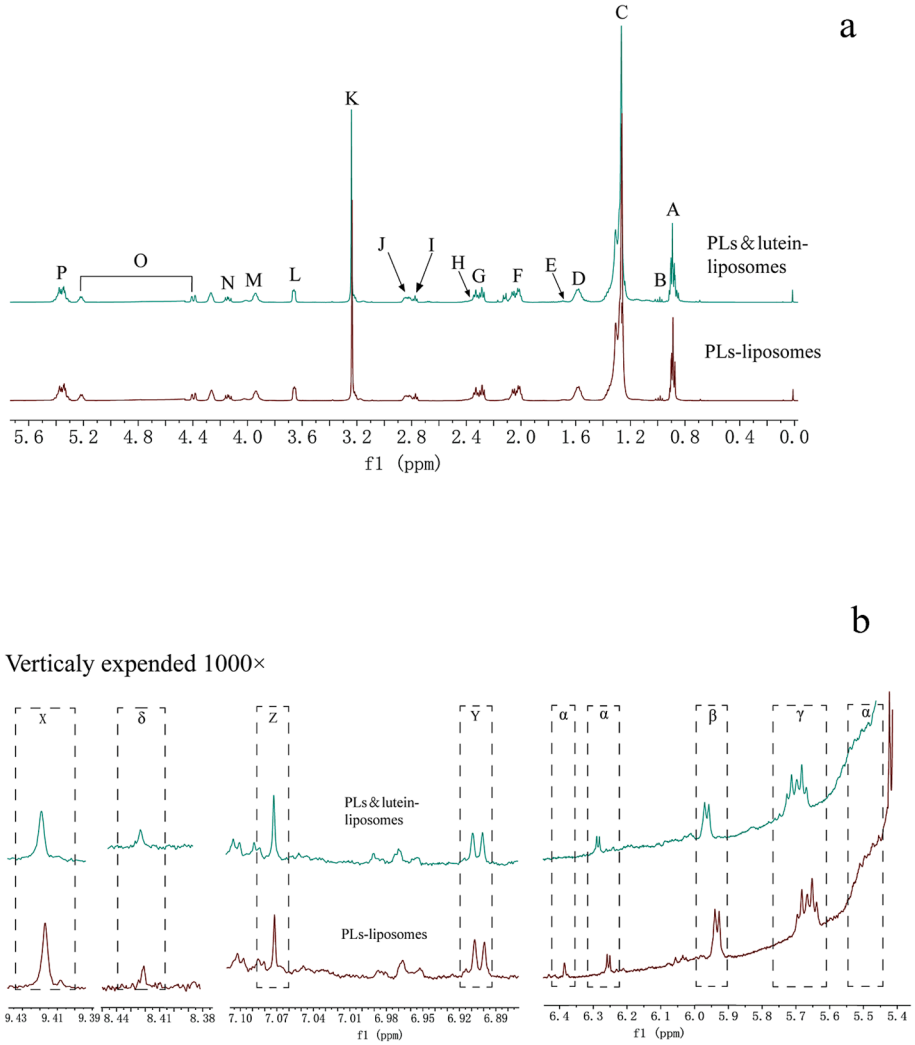

Fig. 1. ^{1}H NMR spectra of heat-treated PLs-liposomes and PLs&lutein-liposomes (a: 0–5.6 ppm ; b: 5.4–9.5 ppm). The regions of 8.2–8.7 ppm and 5.5–7.0 ppm are for hydroperoxides (signals δ) and conjugated dienic systems (signals α, β, γ, Y and Z), respectively.

found in the previous studies: Lutein served as an antioxidant during photooxidation by quenching triplet sensitizer or singlet oxygen (Viljanen, Sundberg, Ohshima, & Heinonen, 2002), so it inhibited the oxidation of lipids; and lutein-phospholipids emulsion could inhibit the oxidation of egg yolk protein, thus improving its physicochemical properties (Yuan et al., 2021).

3.3. ^1H NMR analysis of the oxidation products

The ^1H NMR spectra of the two liposomes in $CDCl_3$ after 10 min of heating are shown in Fig. 1, and the chemical shifts, their corresponding functional group information and labeled numbers are shown in Table 1. Molar percentages (%) of the acyl groups in the two liposomes calculated from the ^1H NMR spectra are shown in Supplementary Table 1. The saturated plus modified (S + M) acyl groups (54.84 %) were the highest among these acyl groups in egg yolk PLs, followed by the oleic or monounsaturated (O or MU) acyl groups (27.80 %) and the linoleic or diunsaturated ω-6 (L or DU) acyl groups (14.37 %). This result was almost identical to the fatty acid composition of egg yolk PLs reported by Zhao et al. (2023). As approximately 12 μg of egg yolk PLs can produce 1 μg of (*E,E*)-2,4-decadienal under ideal conditions, and the threshold value of (*E,E*)-2,4-decadienal is very low (0.07 ng/g) (Chen et al., 2023), very small amount of oxidized PLs could cause strong fatty note. In the present study, the molar percentages of each acyl group were not significantly different in the two samples, because the acyl groups consumed by the oxidation were very small compared to the total number of acyl groups under the current heating condition (100 °C, 10 min). Thus, there was little effect of lutein on the consumption of acyl groups in the present study.

Hydroperoxide and conjugated diene signals were labeled in ^1H NMR spectra (Fig. 1b). The molar concentrations of hydroperoxides in PLs-liposomes were significantly higher than those of PLs&lutein-liposomes, as shown in Table 2, which was consistent with the results of POVs. Meanwhile, the (*E,E*)-conjugated double bond signals bound to hydroperoxy (*OOH*) (*α*) and ketone (*Z*) showed significant differences between the two samples, while there were no significant differences in the (*Z,E*)-conjugated double bond signals (*β* & *γ*). This might be related to their stability. (*E,E*)-Conjugated double bonds were more thermodynamically stable than (*Z,E*)-conjugated double bonds. Therefore, (*Z,E*)-conjugated double bonds were prone to transformation into more stable (*E,E*)-conjugated double bonds (Martin-Rubio et al., 2018). Thus, the content of (*E,E*)-conjugated double bonds (*α* & *Z*) in PLs-liposomes sample was significantly higher than that in PLs&lutein-liposomes sample because of lack of antioxidation of lutein. The number of hydroperoxides was less than the sum of the conjugated diene signals since the conjugated double bond on some of the aldehyde structures also produced signals around 7.0–5.6 ppm (Martin-Rubio et al., 2018).

Some secondary oxidation products, such as aldehyde (signals *X*) and ketone (signals *Y* & *Z*) were identified in the ^1H NMR spectra (Fig. 1b and Table 2). Those secondary oxidation products in PLs-liposomes were significantly higher than those in PLs&lutein-liposomes, which were consistent with the results of POVs, hydroperoxides and (*E,E*)-conjugated double bonds. When primary oxidation compounds were heated, they decomposed into alkoxy radicals, thus forming the secondary oxidation products (Chen et al., 2023; Choe and Min, 2006). Therefore, more primary oxidation compounds were in PLs-liposomes, and more secondary oxidation products were produced. In conclusion, the results of ^1H NMR spectra demonstrated that encapsulation of lutein could lower the molar concentrations of the primary and secondary oxidation products in PLs&lutein-liposomes sample.

3.4. Volatile compounds analyzed by GC–MS

The concentrations of the key volatile compounds in the two liposome samples after heat treatment are shown in Table 3 according to their possible origin. Clearly, most of the lipid-derived products (except

Table 1
Chemical shift distribution and multi peak types of ^1H NMR signals of main acyl groups and oxidation products of egg yolk phospholipids.

Signal	Chemical shift (ppm)	Multi-plicity[1]	Functional group	
			Type of protons	Compound
Main acyl groups				
A	0.88–0.93	t	–C**H**$_3$	saturated/ monounsaturated ω-9 and unsaturated ω-6 acyl groups
B	0.97	t	–C**H**$_3$	unsaturated ω-3 acyl groups
C	1.19–1.33	m	-(C**H**$_2$)$_n$-	acyl groups
D	1.47–1.53	m	–OCO-CH$_2$-C**H**$_2$-	Acyl groups except for DHA, EPA and ARA acyl groups
E	1.59–1.66	m	–OCO-CH$_2$-C**H**$_2$-	EPA and ARA acyl groups
F	1.94–2.14	m	–C**H**$_2$-CH = CH–	Acyl groups except for –CH$_2$- of DHA acyl group in *β*-position in relation to carbonyl group
G	2.22–2.26	dt	–OCO-C**H**$_2$-	Acyl groups except for DHA acyl groups
H	2.31 ~ 2.36	m	–OCO-C**H**$_2$-C**H**$_2$-	DHA acyl groups
I	2.76	t	=HC-C**H**$_2$-CH=	Diunsaturated ω-6 acyl groups
J	2.77–2.80	m	=HC-C**H**$_2$-CH=	Triunsaturated ω-3 acyl groups
K	3.35	s	-N$^+$(C**H**$_3$)$_3$	Phosphatidylcholine
L	3.78	s	CH$_2$C**H**$_2$N$^+$(CH$_3$)$_3$	
M	3.93	m	–C**H**$_2$—O—P-	Phosphatidyl
N	4.10	dtd	-C**H**$_2$OCOR	Glyceryl aroups
O	4.32	dd	ROC**H**$_2$-CH(OR')-C**H**$_2$OR''	Glyceryl aroups
	5.27	m	ROCH$_2$-C**H**(OR')-CH$_2$OR''	
P	5.28–5.46	m	–C**H** = C**H**–	Acyl groups
Oxidation compounds				
α	5.47, 5.76, 6.06, 6.27	ddm, dtm, ddtd, ddm	–C**H** = C**H**-C**H** = C**H**–	(*E,E*)-conjugated double bonds associated with hydroperoxy group in octadecadienoic acyl groups
β	5.40, 5.64, 5.94, 6.45	ddt, dd, dd, ddd	–C**H** = C**H**-C**H** = C**H**–	(*Z,E*)-conjugated double bonds associated with hydroxy group(OH) in octadecadienoic acyl groups
γ	5.51, 5.56, 6.00, 6.58	dtm, ddm, ddtd, dddd	–C**H** = C**H**-C**H** = C**H**–	(*Z,E*)-conjugated double bonds associated with hydroperoxy (OOH) group in octadecadienoic acyl groups
δ	8.3–8.7	bs	–OO**H**	Hydroperoxides
X	9.41	s	–CHO	2,4-Dienal
Y	6.82	m	O=C < C**H** = C**H**–	The double bond conjugated to the ketone group
Z	7.13	dm	–CO-CH = C**H**-CH = CH–	(*E,E*)-conjugated double bonds associated with a keto group

[1] t: triplet; m: mutiplet; d: doublet; bs: broad signal.

(*E*)-2-nonenal, (*E,Z*)-2,4-decadienal, (*E*)-2-decenal and (*E,E*)-2,4-decadienal) and all Maillard reaction products in PLs&lutein-liposomes sample were significantly lower in concentration than those in PLs-liposomes sample. 2-Pentylfuran and hexanal are two essential indicator compounds of ω-6 fatty acids oxidation (Choe & Min, 2006), and their concentrations in PLs-liposomes sample were approximately 9 and 7 times higher than those in PLs&lutein-liposomes sample, respectively, which indicated that PLs-liposomes sample was more prone to oxidation. These two indicator compounds were well correlated with the

Table 2
The contents of oxidation products of PLs-liposomes and PLs&lutein-Liposomes heated at 100 °C for 10 min.

Signal	Functional group	Molar concentration (mmol/mol PL)		sig[1]
		PLs-liposomes	PLs&lutein-liposomes	
α	–CH = CH-CH = CH–	1.17±0.39	0.21±0.01	*
β	–CH = CH-CH = CH–	1.23±0.14	1.11±0.19	ns
γ	–CH = CH-CH = CH–	1.72±0.29	1.84±0.11	ns
δ	–OOH	0.39±0.01	0.29±0.01	*
X	–CHO	2.77±0.21	2.14±0.19	*
Y	O=C < CH = CH–	7.56±0.21	2.96±0.14	*
Z	–CO-CH = CH-CH = CH–	1.03±0.04	0.54±0.06	*

[1] ns means no significant difference,* indicates that the molar concentrations of PLs-liposomes and PLs&lutein-liposomes are significant different at $p = 0.05$.

results of the secondary oxidation products measured by ^{1}H NMR spectra, suggesting that lutein inhibited the thermal oxidation of PLs. However, it was noteworthy that the addition of lutein to the liposomes did not significantly inhibit the formation of (*E,E*)-2,4-decadienal and (*E,Z*)-2,4-decadienal. This might be due to the fact that 2,4-decadienals can further generate 2-octenal and hexanal by retroaldolization (Frankel, 1991), so the increase of their concentrations in PLs-liposomes sample was lower than that of hexanal. Some Maillard reaction products were present in the liposomes after heating, as small amounts of Maillard reaction precursors (reducing sugars and free amino acids) from egg yolk were co-extracted during the extraction of PLs (Ye et al., 2022). The Maillard reaction products, such as 3-methyl butyraldehyde, benzaldehyde and phenylacetaldehyde were significantly reduced in PLs&lutein-liposomes sample, confirming that lutein not only had a protective effect on lipid oxidation, but also inhibited Maillard reaction simultaneously (Yang et al., 2022). The content of *β*-ionone in the PLs&lutein-liposomes sample was 97.7±9.3 ng/sample, which was significantly higher than that in PLs-liposomes sample. As *β*-ionone is a degradation product of lutein (Aparicio-Ruiz et al., 2011), it could be seen clearly that some lutein degraded during the heating process.

3.5. Comparison of liposome samples

The primary and secondary oxidation product content, as well as the concentrations of volatile compounds were used as variables and imported into SIMCA-P14.0 software for model analysis using Orthogonal Partial Least Squares-Discriminant (OPLS-DA). These results are shown in Fig. 2(A). The explanatory rate parameter ($R^2Y = 0.98$) and the predictive power parameter ($Q^2 = 0.95$) indicated that the model had good stability and predictive power. The replacement test of 200 iterations ($R^2 = 0.34$, $Q^2 = -2.53$) indicated that the OPLS-DA model was reliable and did not suffer from overfitting. In the score plot, the same color indicated parallel samples, and different colors represented different samples. When the distribution points are closer, it indicates that the variables included in the statistical analysis are similar, and the reproducibility of the same group of samples is good. Conversely, the sample points farther apart can reflect significant differences between samples. Clearly, it could be seen that the parallel samples were well aggregated, and the separation of the two liposome samples was very high.

The Variable Importance in Projection (*VIP*) in the first principal component of the OPLS-DA further quantifies the contribution of each variable to the differentiation of the samples; the higher *VIP* value, the greater difference in variables different between samples, and a *VIP* >1 is usually indicated as a critical variable. The *VIP* values of the two liposome samples are shown in Fig. 2(B), and the key variables with *VIP* >1 were *β*-ionone, (*E*)-2-Octenal, O=C < CH = CH–, –OOH, hexanal, etc. Where *β*-ionone originated from the decomposition of lutein, the others (except benzaldehyde and phenylacetaldehyde) were lipid-derived products which were strongly affected by lutein, thus demonstrating that lutein was the key reason for the difference between the two liposome samples.

4. Conclusion

It has been demonstrated that lutein can depress the oxidation of PLs-liposomes and inhibit the formation of lipid-derived volatile compounds. Those compounds are the key odorants of chicken meat and fried foods. Therefore, this result has confirmed that the removal of lutein is the determining factor in using egg yolk PLs as an ideal precursor of fatty note odorants.

Table 3
Mean values (n = 4) and identification of the key odorants in headspace of the samples of PLs-liposomes and PLs&lutein-liposomes by HS-SPME-GC–MS.

Compound	LRI DB-WAX[1]	ID[2]	Mean ± SD (ng/sample) [3]		Sig[4]	Possible precursor (Chen, et al., 2023; Chen et al., 2019a)
			PLs-liposomes	PLs&lutein-liposomes		
Hexanal	1086	A	3554.3±679.9	525.9±168.1	**	ω-6 acyl groups
2-Pentyl-furan	1232	A	6398.9±356.7	707.4±102.4	**	ω-6 acyl groups
(*E*)-2-Octenal	1410	A	1601.4±70.7	864.9±448.0	*	ω-6 acyl groups
(*E*)-2-Nonenal	1516	A	539.9±177.9	344.3±69.6	ns	ω-6 acyl groups
1-Octen-3-ol	1445	A	1653.7 ± 469.6	194.3±14.6	*	ω-6 acyl groups
Decanal	1473	A	1250.5±267.6	371.2±160.9	*	ω-9 acyl groups
(*E,E*)-3,5-Octadien-2-one	1549	B	897.9±162.2	195.6±128.1	*	ω-3 acyl groups
(*E*)-2-Decenal	1594	A	1998.4±406.7	1340.5±371.2	ns	ω-9 acyl groups
(*E,E*)-2,4-Nonadienal	1592	A	995.1±310.4	347.2±142.9	*	ω-6 acyl groups
(*E*)-2-Undecenal	1720	A	1530.3±155.3	998.1±175.8	*	ω-9 acyl groups
(*E,Z*)-2,4-Decadienal	1751	B	3725.3±505.7	3610.3±189.4	ns	ω-6 acyl groups
(*E,E*)-2,4-Decadienal	1788	A	6048.6±852.5	4686.1±1023.4	ns	ω-6 acyl groups
3-Methyl-butanal	924	A	176.5±60.6	44.5±40.9	*	Leucine
Benzaldehyde	1492	A	469.1±101.0	92.9±19.1	*	Phenylalanine
Benzeneacetaldehyde	1623	B	421.2±101.6	84.6±20.4	*	Phenylalanine
β-ionone	1953	B	13.2±1.7	97.7±9.3	**	lutein

[1] Linear retention indices determined on a DB-WAX column.
[2] Confirmaton of identity, where A = mass spectrum and LRI agree with those of the authentic compound; B = mass spectrum agrees with reference spectrum in the NIST mass spectral database and the LRI value of WAX agrees with that in the database (NIST Chemistry WebBook, 2018).
[3] Approximate amount (mean, n = 4) collected from the headspace, calculated by comparison of peak area with that of 1,2-dichlorobenzene (130.6 ng) with a response factor of 1. Analysis of variance was performed on the samples within PLs-liposomes or PLs&lutein-liposomes, respectively.
[4] Sig, obtained from a *T*-Ttest that there is a difference between means; ns = no significant difference between means; * and ** indicate significant differences at $p < 0.05$ and $p < 0.01$, respectively.

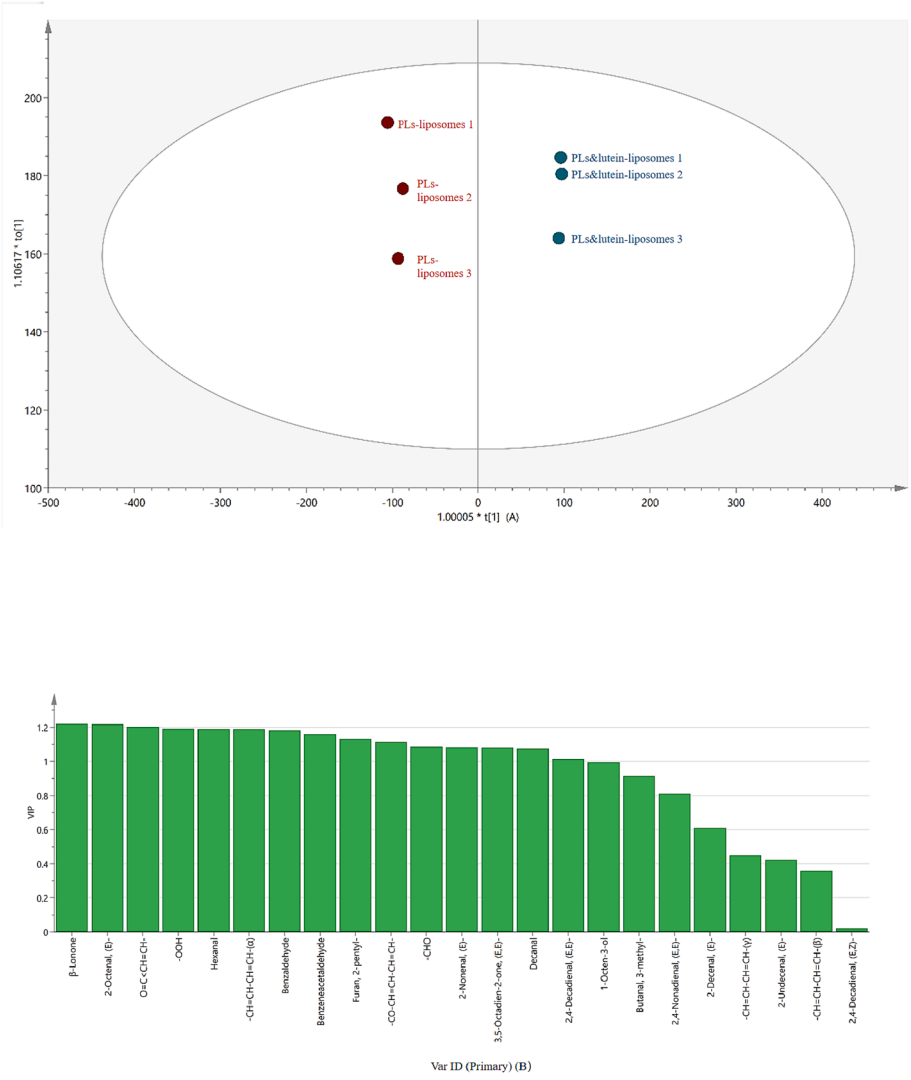

Fig. 2. Orthogonal partial least squares-discriminant analysis score scatter plot (A) and variable important in projection values of the products in PLs-liposomes and PLs&lutein-liposomes (B).

Declaration of interest

The authors declare that they have no conflict of interest.

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.

Data availability

Data will be made available on request.

Acknowledgments

This work was funded by "National Natural Science Foundation of China (No. 31960513)".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100945.

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