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Comprehensive lipidomics and flavoromics analysis reveals the formation mechanism of the unique flavor in quail egg yolks during thermal treatment

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Abstract

After thermal treatment, quail eggs pruduce an attractive flavor that is favored by consumers. In this study, the key aroma volatiles and their lipid precursors during thermal treatment were investigated by the electronic nose, GC-MS, GC-O-MS and UPLC-Q-Exactive HF-X. The results exhibited that the raw and fresh flavor of raw egg yolks came from 1-Octen-3-ol, 2-Ethyl-1-hexanol, 1-Decene, and 1-Undecene. 2-Methyl-3-octanone, Toluene, and some aromatic compounds worked synergistically to contribute to the aroma profile of boiled eggs. (+)-2-Bornanone, Octanal, 2-Methyl-butanal, Nonanal, (.+Dihydro-3-hydroxy-4,4-dimethyl-2(3 H)-furanone, 6,7-Dihydro-2,5-dimethyl-5 H-cyclopentapyrazine, (E)-2-methyl-6-(1-propenyl)-pyrazine, 2-Ethyl-3,5-dimethyl-pyrazine, 3,5-Diethyl-2-methyl-pyrazine, 2,5-Dimethyl-pyrazine were the key flavor compounds in the fried egg and gave it the popcorn and roasted flavors. The statistical analysis of the lipid profile revealed that brief, high-temperature heating (100 or 200 °C for 10 min) in typical boiled and fried quail eggs had minor effects on the lipid nutritional value. PE-related lipids, particularly those containing 18-carbon fatty acids, contributed to the aroma formation of fried quail eggs.

Keywords Quail egg yolk, Flavoromics, Key aroma volatiles, Lipid oxidation, Lipidomics, PE-related lipids

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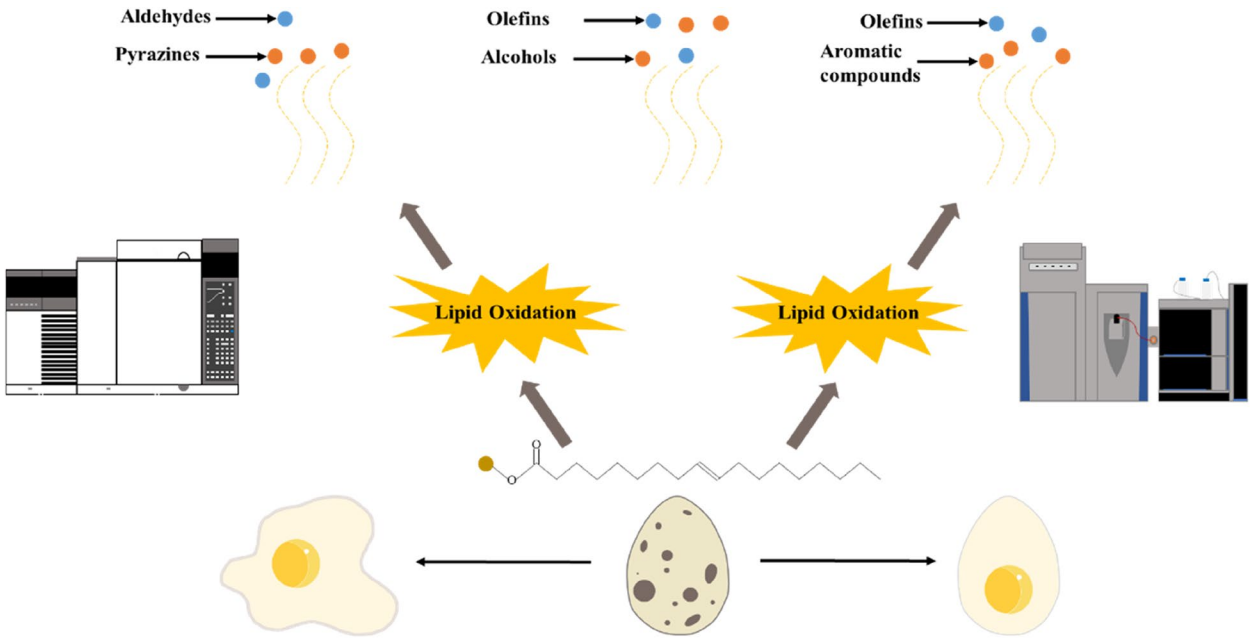
Graphical Abstract



Introduction

Eggs are a kind of animal-derived food with low price, delicious flavor and rich nutrition, containing essential fatty acids, amino acids, minerals, vitamins and high-quality protein (Benelam et al. 2012). Quail eggs are popular eggs in Brazil and many Asian countries (such as Japan). It possessed higher nutritional value than eggs (Bao et al. 2020; Sun et al. 2019), referred to as "the ginseng of animals" (Santos et al. 2015). Regular consumption of quail eggs can enhance immunity, promote brain activity, improve memory health, and stabilize the nervous system (Liu et al. 2020). Heating treatment, such as frying and boiling, is the typical processing method of quail eggs, giving the products umami, kokumi and tender flavor and making it highly desirable. According to several studies, the flavor of eggs during thermal treatment is generated by the lipids in the yolk (Koehler & Jacobson 1966), where almost all of the lipids of eggs are concentrated. These lipids are mainly in the form of lipoproteins (Sugino et al. 2018). Although the flavor of quail egg products is very interesting, only a few studies have been performed on egg flavor, which only focused on the flavor of raw eggs (Wang et al. 2014). There is limited research on the key flavor compounds and their formation mechanisms in quail egg yolks during heating treatments, which is an area of great interest.

Lipid oxidation plays a critical role in the development of heat-processed food flavor, particularly those in animal products that undergo baking, drying, boiling, and frying treatments (Bi et al. 2023; Qiu et al. 2023; Zhang et al. 2022). Generally, lipid oxidation is responsible for the formation of more than half of the volatile compounds in animal foods, and aldehydes, ketones, alcohols, acids, esters, etc. produced by lipid oxidation are often thought to cause the aroma characteristic of these foods (Bravo-Lamas et al. 2018; Wang et al. 2022). For example, aldehydes and alcohols are the highest volatiles in boiled lamb, mainly derived from the oxidation of unsaturated fatty acids during cooking (Bravo-Lamas et al. 2018). Yu et al. found that the main volatile flavor compounds in boiled, fried and roasted chicken were phenylacetaldehyde, 3-butanedione and 3-methylbutyraldehyde, respectively, which were associated with lipid oxidation (Yu et al. 2021). In addition, the coordination of lipid oxidation and amino acids also affects the flavor of food (Hidalgo & Zamora 2019; Qiu et al. 2023). For example, pyrazines are among the most important compound class conveying the odor impressions "roasty", "nutty", and "earthy" (Liang et al. 2022). They are often thought to be formed by the Maillard reaction. However, some studies found that dicarbonyl compounds produced by lipid oxidation also participate in pyrazine formation coordinating the Maillard reaction (Gao et al. 2023; Zamora

& Hidalgo 2005; Zhao et al. 2019). Therefore, lipids are important flavor precursors for heat-processed food, while the relationship between flavor and lipids in quail egg yolk during thermal treatment is worth exploring.

During food processing, many factors can change the flavor of food, and temperature is one of the most important factors. It can change the type and content of volatile compounds by influencing lipid oxidation and Maillard reaction, leading to the difference in flavor profile in food (Zhou et al. 2022a, b). Qiu et al. discovered that different heating temperatures affected the lipid oxidation in semi-dried golden pompano, resulting in the difference of its flavor, and low temperature increased the content of aldehydes and generated better flavor (Qiu et al. 2023). The difference in processing methods is largely reflected in the choice of temperature. During the boiling treatment, the processing temperature of food is about 100 °C, but during the frying or baking treatment, the temperature is usually 140–200 °C (Choe & Min 2007). The consumption of boiled and fried quail egg products is widespread, but the knowledge about their flavor profiles and origins is limited and needs further study.

In this study, the key flavor compounds of boiled quail egg yolks (heated at 100 °C, which is a common boiling temperature) and fried egg yolks (heated at 200 °C, which is a common frying temperature) and their relationship with lipid oxidation were explored. The flavor profile, volatile compounds and lipid molecular species were monitored by the electronic nose, gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry-mass spectrometry (GC-O-MS) and ultra-high performance liquid chromatography-Q-Exactive HF-X orbitrap mass spectrometer (UPLC-Q-Exactive HF-X). The key flavor compounds of common quail egg products were identified by the analysis of electronic nose, GC-MS and GC-O-MS data. The lipid changes in egg yolk during thermal treatment were compared, and important flavor precursors were concerned and explored through the analysis of lipids and lipid oxidation products. This study reveals the key flavor compounds and their lipid precursors of the common quail egg products, providing a theoretical foundation for the development of quail egg flavor-related products.

Materials and methods

Materials and chemicals

Ammonium formate and cyclohexanone were purchased from Sigma-Aldrich (Madison, USA). HPLC-grade solvents (methanol, chloroform, isopropanol and acetonitrile) were purchased from Spectrum Chemical Mfg. Corp (Gardena, CA, USA). PC 17:0/17:0 (≥99%), PE 17:0/17:0 (≥99%) and PG 17:0/17:0 (≥99%) were obtained from Aladdin Reagent Co., Ltd (Shanghai, China), LPC 17:0 (≥99%) was bought from Avanti Polar Lipids (Alabama, USA), and TAG 17:0/17:0/17:0 (≥99%) were purchased from Sigma-Aldrich (Madison, WI, USA).

Two hundred fresh quail eggs were bought from a local market in Hebi, China. The variety and source of the quail egg were the same. The weight of each quail egg was around 10 g. The quail eggs had a light brown shell with black-brown speckles.

Samples pretreatment at different temperatures

The egg yolks, from 30 specimens, were manually separated from the egg shells and whites. Egg yolks (2 g) were taken in a 20 mL brown sample bottle and heated at 100 and 200 °C for 10 min using an oil bath. The bottle was sealed to avoid losing volatiles. Unheated samples were set as the control samples. After heating, the samples were promptly chilled with ice for 10 min and then analyzed by the electronic nose, GC-MS, and UPLC-QE-MS/MS immediately. All assays were performed in triplicate.

Electronic nose analysis

Electronic nose analysis can simulate human senses via a sensor array system and be used to detect volatile components rapidly. The pretreatment samples were incubated via a water bath at 50 °C for 10 min. Then, A PEN 3 electronic nose (Win Muster Airsense Analytics Inc., Schwerin, Germany) was used to analyze the egg yolk samples. During the test, a hollow needle attached to the tubing was utilized to puncture the seal of the vial and draw out the volatile gases. To replace the volatile gases, fresh air was introduced through a second hollow needle equipped with a charcoal filter. The measurement time was set as 60 s and a standby procedure was set as 100 s to ensure that the sensor signals returned to their baseline levels.

Ten metal oxide semiconductors were utilized in the electronic nose for specific identification of different volatile classes. Details of each semiconductor are shown in Table S1.

Volatile compounds analysis using GC-MS analysis

Volatile compounds in the pretreatment yolk samples were analyzed by GC-MS (GC7890b, MS5977a MSD, Santa Clara, CA) using the VF-WAXms capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies Inc., Palo Alto, CA). The yolk in the heated sample was mashed with a small spoon, and the raw yolk sample was covered with the walls of the bottle to release more volatile compounds. The samples were added with a cyclohexanone internal standard (50 μg/mL, 3 μL), incubated for 20 min at 60 °C, and then adsorbed by SPME fiber (coated with PDMS/DVB/CAR) for 40 min at 60 °C (Zhou et al. 2019). A VF-WAXms column was used to

separate volatile compounds. The inlet temperature was 250 °C. The GC oven temperature program initiated at 35 °C and gradually increased at a rate of 3 °C/min until it reached 75 °C, held for 3 min, then ramped up to 240 °C at a rate of 5 °C/min with another 5 min hold. EI source was set as 70 ev. Helium was used as the carrier gas at a flow rate of l.68 mL/min. Scan mode (m/z 50–550) was used for mass selective detection.

The mass spectra of volatile compounds were compared to NIST14 reference spectra for identification, using retention index (RI) and reverse matching factor (similarity > 700). Linear retention indices of volatiles were calculated based on n-Alkanes (C_6-C_{30}) (Huang et al. 2019).

GC-O-MS analysis

Characterization of odor-active volatile compounds was performed using a GC-MS system coupled with a sniffing port (ODP3; Gerstel, Mülheim an der Ruhr, Germany) and a VF-WAXms column (30 m × 0.25 mm × 0.25 μm). The transfer line to the sniffing port was kept at 240 °C, and humidified air was introduced into the port at a flow rate of 60 mL/min. Sample pretreatment was carried out as described for GC-MS detection. Volatile compounds were separated on a VF-WAXms column using a temperature program that started at 40 °C for 2 min, followed by a ramp of 6 °C/min until 240 °C, and held for 5 min. The inlet temperature was set at 250 °C, and helium was used as the carrier gas at a flow rate of 1.68 mL/min. Mass selective detection was performed in scan mode, scanning from m/z 50 to 550 with an electron ionization (EI) source set at 70 ev. Olfactory analysis was conducted by three judges, with a total of nine GC-O analyses performed, each judge conducting three analyses.

Lipidomics analysis using UPLC-Q-Exactive HF-X

Lipid in quail egg yolk was extracted with the Folch method (Zhang et al. 2018). Deionized water (5 mL) was mixed with the pretreatment samples (0.5 g). The sample was homogenized and then supplemented with chloroform/methanol (1.875 mL, 2:1, v/v). After shaking and centrifugation at 8000 rpm for 10 min, the organic layers were collected in pre-weighed vials and dried with a centrifuge concentrator. Then, the dried samples were re-dissolved in methanol as 10 mg/mL redissolution. A combination of internal standards (PE 17:0/17:0, PC 17:0/17:0, PG 17:0/17:0, LPC 17:0, TAG 17:0/17:0/17:0) (30 μL, 100 μg/mL), resolved sample (30 μL), and methanol (240 μL) were vortexed for 6 min to dissolve the lipids before they went through further purification by centrifugation at 14,000 rpm for 10 min (Tomas & Oliver 2016; Zhang et al. 2019). All samples were immediately detected by UPLC-Q-Exactive HF-X.

The lipid molecular species were analyzed via UPLC-Q-Exactive HF-X (Thermo Fisher, CA). The liquid chromatography conditions were as follows: analytes were separated by an Acquity UPLC BEH C8 column (2.1 mm×100 mm; 1.7 μm) with an Acquity BEH C8 VanGuard precolumn (2.1 mm×5 mm; 1.7 μm) (Waters, MA, USA); Mobile phase (A) was acetonitrile/water (6:4, v/v) and Mobile phase (B) was isopropanol/acetonitrile (9:1, v/v) with 10 mmol/L ammonium formate (Zhang et al. 2019); The column temperature was maintained at 65 °C; The flow rate was 0.6 mL/min. The following gradient elution was used: from 0 to 2 min, mobile phase B was increased from 15 to 30%; from 2 to 2.5 min, B was increased from 30 to 48%; from 2.5 to 11 min, B was increased from 48 to 82%; from 11 to 11.5 min, B was increased from 82 to 99%; from 11.5 to 12 min, B remained at 99%; from 12 to 12.1 min, B was lowered from 99 to 15%; and from 12.1 to 15 min, the percentage of B remained at 15% (Zhang et al. 2019).

The MS/MS instrument utilized the ESI (+)/(−) modes for Full MS/ddMS. The electrospray ionization (ESI) source parameters: sweep gas flow rate was 2%, sheath gas flow rate was 60%, auxiliary gas flow rate was 25%, auxiliary gas heater temperature was 370 °C, the capillary temperature was set at 380 °C, and a spray voltage of 3.6 kV was applied in positive ion mode while 3.0 kV was used in negative ion mode.

Non-targeted lipidomics data was utilized to manually annotate oxidized lipids referring to our previous methods (Zhou et al. 2022a). Firstly, oxidation products were predicted according to common lipid oxidation mechanisms (Xia & Budge 2017). The m/z of precursor ions and product ions of oxidized lipids were calculated in accordance with lipid fragmentation rules in the Lipidomics Standards Initiative (LSI) guidelines. Then, referring to the calculated mass spectrum information of oxidized lipids, we searched the corresponding precursor ions of oxidized lipids in MSDAIL and check their product ions in the mass spectra (Grüneis et al. 2019; Petronilho et al. 2021). Finally, three oxidized phosphatidylcholine (oxidized PC) and three oxidized phosphoethanolamine (oxidized PE) were identified.

Statistical analysis

MS-DIAL was used to identify the volatiles and lipid molecular species (Tsugawa et al. 2015). SPSS 26.0 (SPSS Inc. USA) was employed to perform significance analysis on both volatile and lipid data. The one-way ANOVA test ($P < 0.05$) was used to evaluate differences between samples, and the Tukey method was applied for post-hoc

analysis of significant differences. MetaboAnalyst 4.0 was utilized for partial least squares discriminant analysis (PLS-DA), principal component analysis (PCA), variable importance in projection (VIP) analysis, and significance analysis of metabolomics (SAM) (Chong et al. 2018). To enhance readability due to the abundance of data points in the loading plot, we performed additional processing on the data exported from MetaboAnalyst 4.0 in Excel. OmicStudio was utilized for correlation heatmap between volatile compounds and lipid molecular species (Lyu et al. 2023).

Results and discussion

Analysis of volatile flavor compounds in quail egg yolk

To investigate the characteristic flavor of quail eggs during boiling and frying, electronic nose and GC-MS were used to monitor their flavor profiles after heating at 100 and 200 °C for 10 min, respectively (They simulate boiled egg yolks and fried egg yolks respectively) (Fig. 1). Each of the 10 sensors of the electronic nose is sensitive to a different type of flavor compounds (Table S1) and their results for the flavor profile of quail egg yolk are shown in Fig. 1A. The response values of W5S, W1S, W1W and W2W in the 200 °C-heated egg yolks were higher than the other two, suggesting that fried egg yolks may contain higher levels of nitrogen oxides, methane, sulphur compounds, pyrazines, terpenes, aromatic components and sulphur-containing compounds. The overall flavor profile of the 100 °C-heated egg yolks was close to that of the raw egg yolks, and their response value was close to 1, indicating that both of them had less flavor. The 100 °C-heated egg yolks possessed a higher response at W1C than the other two, indicating that some characteristic aromatic compounds were formed in the boiled egg yolk. The response values of W5S, W1S, W2S and W3S in the raw egg yolks were slightly higher than those of the 100 °C-heated egg yolks, indicating that raw egg yolks possessed more nitrogen oxides, methane, ethanol, aromatic compounds, etc.

A total of 79 volatile compounds were detected by GC-MS in the quail egg yolk samples, with the most diverse alkanes, pyrazines and aldehydes (Fig. 3B). PLS-DA was carried out to analyze GC-MS data as shown in Fig. 3C. The total variance contribution rate of the two principal components reached 77.8%, indicating that it could cover most volatile data information of the samples (Salum et al. 2017). Three egg yolk samples were clustered in different areas of the score plot, indicating that their volatile compounds had clear differences. The loading plot showed the distribution of volatile compounds, where they could be divided into four groups according to the scores plot (Fig. 1D). The first group was distributed in the third quadrant, mainly including alkanes and olefins, corresponding to the unheated egg yolks. The second group distributed in the second quadrant, mainly including benzene and olefins, and they possessed higher levels in the 100 °C-heated egg yolks. The third group mainly included olefins, aldehydes, furans, and benzene, and distributed in the first quadrant and located between boiled and fried egg yolk samples. The fourth group distributed in the fourth quadrant and included mainly aldehydes, pyrazines, ketones and other compounds, which had higher contents in the 200 °C-heated egg yolks.

To explore the key flavor compounds of quail egg yolks at different temperatures, VIP analysis was performed for volatile data (Fig. 1E). VIP is a weighted sum of squares of the PLS-DA loadings considering the amount of explained Y-variation in each dimension. 31 important volatiles (VIP > 1.0) were listed in Fig. 1E and the number of different types of volatiles was shown in Fig. 1F. It is worth noting that although the molecular species of alkanes was the most among these volatiles, only two possessed a VIP greater than 1. The 10/13 of pyrazine compounds had a VIP greater than 1, and their contents all reached the highest level in the 200 °C-heated egg yolks. In addition, four aldehyde compounds with a VIP greater than 1 also had the highest content in the 200 °C-heated egg yolks. Two ketones had a VIP greater than 1. (+)-2-bornanone reached its highest level at the 200 °C-heated egg yolks, but 2-methyl-3-octanone reached its highest level at 100 °C-heated egg yolks. The content of aromatic compounds containing benzene rings and partial olefins had the highest levels in the 100 °C-heated egg yolks. Most alkanes, olefins and alcohols were most abundant in raw egg yolks. Therefore, furans, pyrazines and aldehydes were important volatiles associated with omelette flavor, while aromatic compounds and some olefins contributed a lot to boiled egg flavor. This is consistent with the results of the electronic nose in Fig. 1A. Aromatic compounds may be related to Strecker degradation in the Maillard reaction (Cao et al. 2017). Aldehydes, furans and ketones were common products of lipid oxidation (Zhou et al. 2022a). The pyrazines with high contents in the fried egg yolks may be related to both the Maillard reaction and lipid oxidation. Some studies have shown that ammonia or amino acids react with α-dicarbonyl compounds to form α-aminocarbonyl compounds, which is a precursor to pyrazine formation. While lipids are an important source of α-dicarbonyl compounds (Zamora & Hidalgo 2005). Negroni et al. found that the presence of lipid oxidation products greatly affected pyrazine formation in model systems containing vegetable oils, lysine, xylose, and glucose (Negroni et al. 2001). Therefore, as a high-fat food, the significant formation of pyrazine compounds in egg yolk may be closely connected to lipid oxidation.

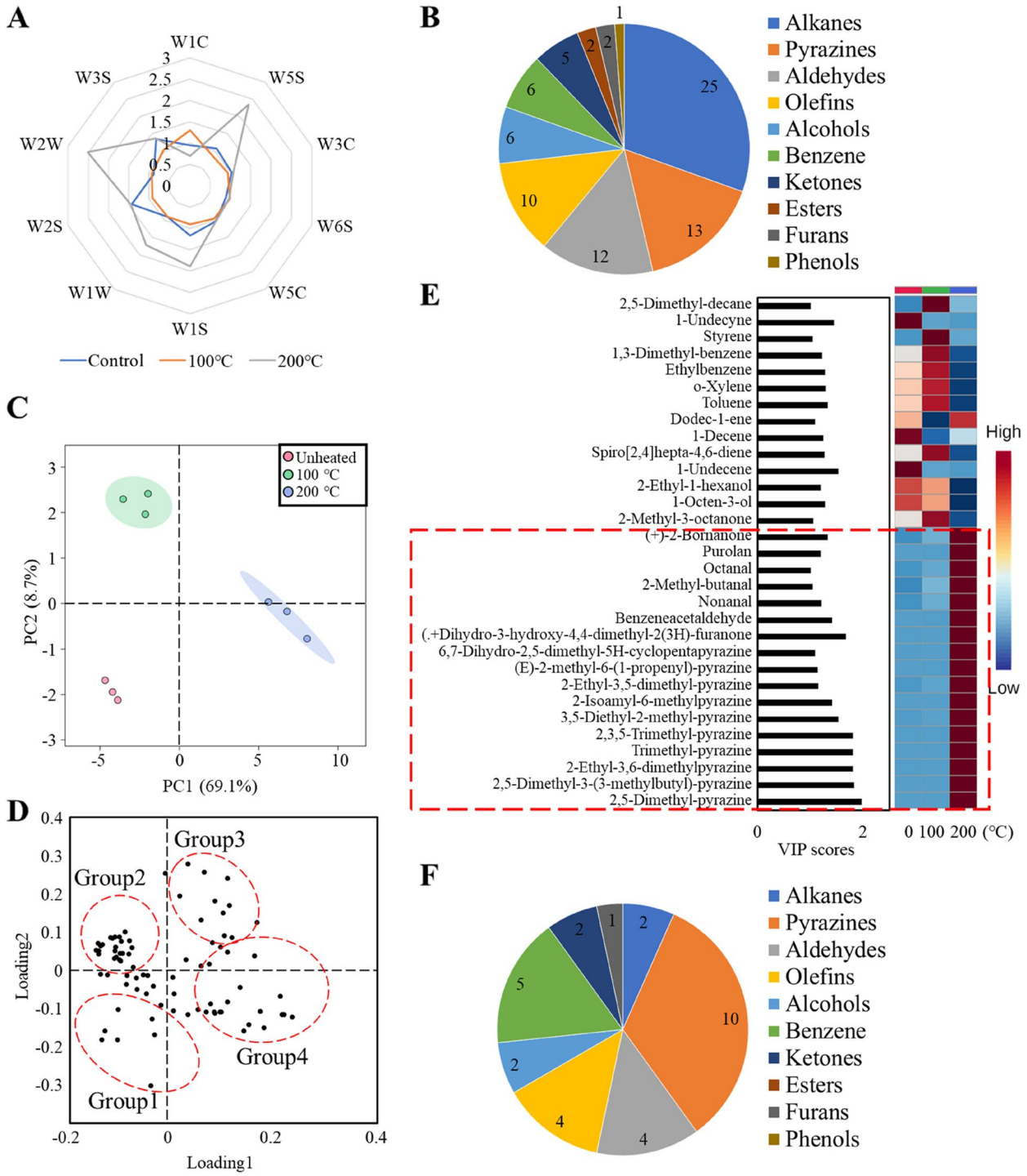


Fig. 1 Analysis of volatiles of quail egg yolk during thermal treatment. **A** Electronic nose analysis; **B** Category comparison of 71 volatiles; **C** Scores plot based on PLS-DA; **D** Loading plot based on PLS-DA; **E** Heatmap analysis of 31 volatiles with VIP > 1; **F** Category comparison of 31 volatiles with VIP > 1. The numbers in the pie chart **B**, **F** represented the number of volatile compounds in different categories. All assays were performed in triplicate

A

Retention time(min)	Flavor description
3.063	Like cook the meat
5.114	Oil oxidation ordour
9.419	Popcorn flavor
11.894	Stink
12.071	Chewing gum
12.478	The smell of cooked food
13.314	Roasted corn flavor, sweet
13.489	Slight stink
15.081	A slight stink and sour smell
15.251	Tea aroma
15.637	Popcorn, sweet roasted flavor
16.794	Roasted aroma, organic rubber smell
19.095	Roast

B

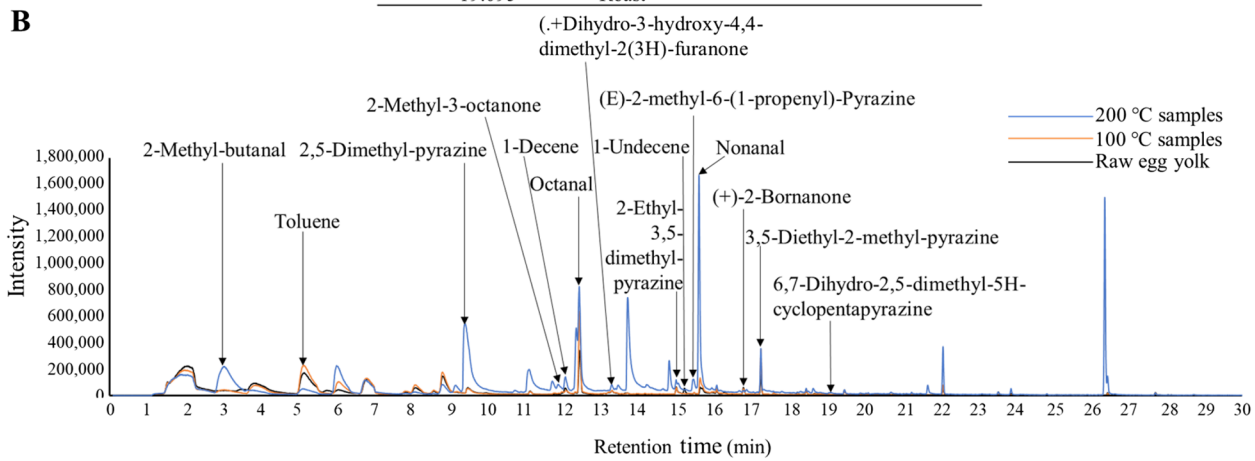


Fig. 2 Comparison and change of lipid molecular species in quail egg yolk. **A** The number of different types of lipids; **B** The content of different types of lipids; **C** Scores plot and **D** Loading plot based on PCA. Different colors and shapes of loading plot represent different types of lipids. All assays were performed in triplicate

The relevant flavor descriptions of 31 volatiles with VIPs greater than 1 were collected in Table 1, where seven volatiles were not clear. Most pyrazines and aldehydes had a clear flavor description and contribute more to the flavor of the fried egg yolk. Pyrazines gave the fried egg a baked and roasted flavor, while aldehydes gave it a fatty flavor (Acree & Arn 2007; Adams et al. 2002; Assoc. 2023; Burdock 2016; Kim et al. 2023). Aromatic compounds containing benzene rings, mainly presented the taste of paint and gasoline (Acree & Arn 2007; Kim et al. 2023), but o-Xylene was different from several other aromatic compounds (Acree & Arn 2007), presenting the taste of geranium. These volatiles worked synergistically and contributed to the flavor of boiled eggs. The raw flavor of raw egg yolks may come from 1-Octen-3-ol, 2-Ethyl-1-hexanol, and 1-Undecene (Assoc. 2023; Kim et al. 2023).

We also performed GC-O-MS analysis on the egg yolk samples, and the results are shown in Fig. 2. The judge sniffed and recorded the relevant flavors in real-time during the GC-O-MS analysis. The flavor descriptions are presented in Fig. 2A. The retention times of volatile compounds with VIP greater than 1 were compared to the time at which the corresponding flavors were perceived, and the flavor compounds were marked on the chromatogram (Fig. 2B). The fried egg flavor was more pronounced, and a large number of related flavors were detected during the GC-O-MS analysis. (+)-2-Bornanone, Octanal, 2-Methyl-butanal, Nonanal, (.+Dihydro-3-hydroxy-4,4-dimethyl-2(3 H)-furanone, 6,7-Dihydro-2,5-dimethyl-5 H-cyclopentapyrazine, (E)-2-methyl-6-(1-propenyl)-pyrazine, 2-Ethyl-3,5-dimethyl-pyrazine, 3,5-Diethyl-2-methyl-pyrazine, and 2,5-Dimethyl-pyrazine were identified as the volatile compounds corresponding to the time points when the flavors appeared. Their concentrations in fried eggs were significantly higher than in the other two types of egg products, indicating that they may be the key flavor substances in fried eggs. These volatile compounds mainly contribute to the popcorn and roasted flavors. The flavor described by sniffing is also consistent with the flavor description of these volatiles in flavor-related websites (Table 1) (Acree & Arn 2007; Adams et al. 2002; Assoc. 2023; Kim

Table 1 RI, molecular formula, odor, identification method, extraction method, references of 31 important volatiles with VIP > 1 generated from quail egg yolk during thermal treatment

No.[a]	Compounds	RI[b]	Molecular formula	Odor[c]	Identification method	Extraction method	References[d]
1	2,5-Dimethyl-decane	1112	C12H26	--	MS/RI	SPME	
2	1-Undecyne	1125	C11H20	--	MS/RI	SPME	
3	Styrene	893	C8H8	Balsamic, gasoline	MS/RI	SPME	(Acree & Arn 2007)
4	1,3-Dimethyl-benzene	866	C8H10	--	MS/RI	SPME	
5	Ethylbenzene	855	C8H10	Like gasoline, an aromatic odor	MS/RI	SPME	(Kim et al. 2023)
6	o-Xylene	887	C8H10	Geranium	MS/RI	SPME	(Acree & Arn 2007)
7	Toluene	763	C7H8	Paint	MS/RI	SPME	(Acree & Arn 2007)
8	Dodec-1-ene	1191	C12H24	Volatiles released during oil heating, a pleasant odor.	MS/RI	SPME	(Kim et al. 2023)
9	1-Decene	989	C10H20	Volatiles released during oil heating, a pleasant odor.	MS/RI	SPME	(Kim et al. 2023)
10	Spiro[2,4]hepta-4,6-diene	759	C7H8	--	MS/RI	SPME	
11	1-Undecene	1091	C11H22	Slight odor, often in heated oil	MS/RI	SPME	(Kim et al. 2023)
12	2-Ethyl-1-hexanol	1030	C8H18O	Green, rose	MS/RI	SPME	(Assoc. 2023)
13	1-Octen-3-ol	980	C8H16O	Cucumber, earth, fat, floral, mushroom	MS/RI	SPME	(Assoc. 2023)
14	2-Methyl-3-octanone	985	C9H18O	Correlation with flavor characteristics produced by lipid oxidation in Tibetan yak meat	MS/RI	SPME	(Hawashin et al. 2016)
15	(+)-2-Bornanone	1143	C10H16O	A warm minty, almost ethereal diffusive aroma	MS/RI	SPME	(Burdock 2016)
16	Purolan	993	C12H26	--	MS/RI	SPME	
17	Octanal	1003	C8H16O	Fat, soap, lemon, green	MS/RI	SPME	(Acree & Arn 2007)
18	2-Methyl-butanal	662	C5H10O	Almond, cocoa, fermented, hazelnut, malt	MS/RI	SPME	(Assoc. 2023)
19	Nonanal	1104	C9H18O	Fat, citrus, green	MS/RI	SPME	(Acree & Arn 2007)
20	Benzeneacetaldehyde	1045	C8H8O	Has a harsh, green odor reminiscent of hyacinth on dilution.It has an unpleasant, pungent, bitter flavor, turning sweet and fruit-like at low levels.	MS/RI	SPME	(Burdock 2016)
21	(.+Dihydro-3-hydroxy-4,4-dimethyl-2(3 H)-furanone	1032	C6H10O3	--	MS/RI	SPME	(Kim et al. 2023)
22	6,7-Dihydro-2,5-dimethyl-5 H-cyclopentapyrazine	1225	C9H12N2	Nuts	MS/RI	SPME	(Adams et al. 2002)
23	(E)-2-methyl-6-(1-propenyl)-pyrazine	1107	C8H10N2	A natural product found in Nicotiana tabacum	MS/RI	SPME	(Kim et al. 2023)
24	2-Ethyl-3,5-dimethyl-pyrazine	1084	C8H12N2	Broth, earth, potato, roast	MS/RI	SPME	(Adams et al. 2002)
25	2-Isoamyl-6-methylpyrazine	1249	C10H16N2	Respiratory tract irritation	MS/RI	SPME	(Kim et al. 2023)
26	3,5-Diethyl-2-methyl-pyrazine	1162	C9H14N2	Baked, cocoa, roast, rum	MS/RI	SPME	(Adams et al. 2002)
27	2,3,5-Trimethyl-pyrazine	1002	C7H10N2	Roast, potato, must	MS/RI	SPME	(Acree & Arn 2007)
28	Trimethyl-pyrazine	1004	C7H10N2	Roast, potato, must	MS/RI	SPME	(Acree & Arn 2007)
29	2-Ethyl-3,6-dimethylpyrazine	1079	C8H12N2	Broth, earth, potato, roast	MS/RI	SPME	(Adams et al. 2002)
30	2,5-Dimethyl-3-(3-methylbutyl)-pyrazine	1315	C11H18N2	--	MS/RI	SPME	
31	2,5-Dimethyl-pyrazine	917	C6H8N2	Cocoa, roast beef, roasted nut	MS/RI	SPME	(Adams et al. 2002)

[a] Numbering refers to the order of the heatmap in Fig. 1E. [b]Retention index determined using a homologous series of n-alkanes. [c]Odor are found in literature or websites. [d]References that provide flavor descriptions

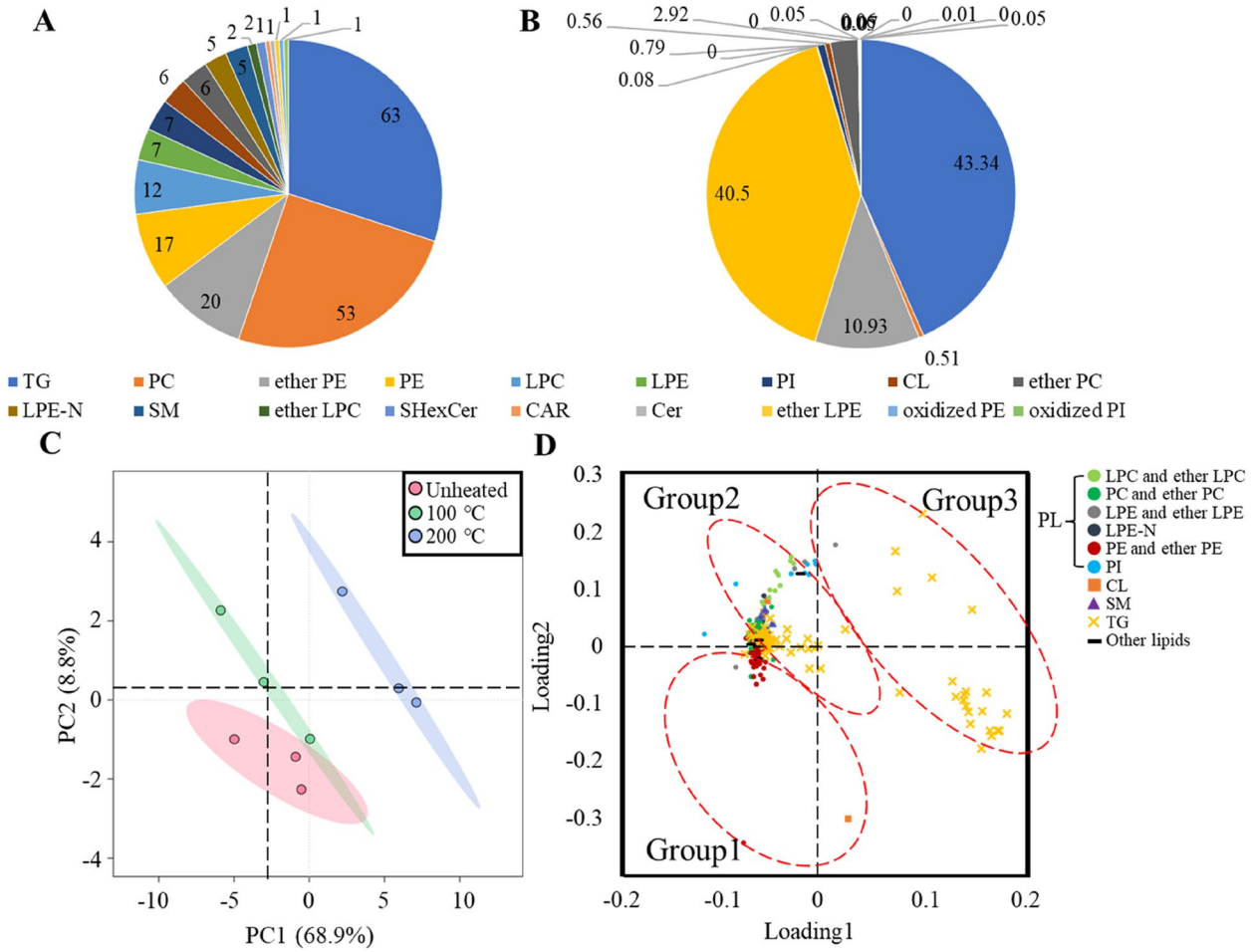


Fig. 3 Analysis of key volatiles of quail egg yolk during thermal treatment via GC-O-MS data. **A** Judges' description of egg yolk flavor in GC-O-MS analysis; **B** Chromatograms of volatile flavor compounds in three egg yolk products and volatile compounds corresponding to important flavor formation time points. All assays were performed in triplicate

et al. 2023). This further confirms that these volatile compounds were the key flavor compounds of fried egg yolks. The retention times of 2-methyl-3-Octanone and Toluene were similar to the flavors of cooked meat and oil oxidation in boiled egg yolk samples, and their concentrations were relatively high in boiled eggs, suggesting their important impact on the boiled egg flavor. 1-Undecene and 1-Decene had higher concentrations in raw eggs and corresponded to the flavors of tea and chewing gum. According to the references, they represent a pleasant odor, and paint odor, respectively (Table 1) (Kim et al. 2023), indicating their significant influence on the flavor of raw eggs. Since the flavors of raw and boiled eggs were relatively less pronounced compared to fried eggs, fewer odors were perceived during the GC-O-MS for raw and boiled egg samples. For the key flavor substances of boiled and fried eggs, a comprehensive analysis combining GC-O-MS and VIP analysis of volatile compounds can be conducted. The flavor of raw eggs may be attributed to 1-Octen-3-ol, 2-Ethyl-1-hexanol, 1-Decene, and 1-Undecene. 2-Methyl-3-octanone, Toluene, and some aromatic compounds worked synergistically to contribute to the aroma profile of boiled eggs.

Analysis of lipid molecular species in quail egg yolk

The quail egg yolk lipid composition was determined by UPLC-Q-Exactive HF-X on full MS/ddMS mode; 210 lipid molecular species (Table S2) from 18 subclasses were identified, mainly including TAG (triglyceride), PC (phosphatidylcholine), PE (phosphoethanolamine), ether phosphoethanolamine (ether PE) et al. Figure 3A shows the number and content of 18 lipid subclasses. Among them, only TAG, PC, ether PE, PE and lysophosphatidylcholine (LPC) had more than 10 molecular species, and the contents of different lipid subclasses were not completely in line with their number, and the lipids with content greater than 1% were TAG, PE, ether PE

and ether PC (ether phosphatidylcholine). PCA was carried out for lipid molecular species data (Fig. 3B and C). The total variance contribution of PC 1 and PC 2 reached 77.7%. The three samples were clearly distinguished in the scores plot. The loading plot could be divided into three groups by referring to the score plot. The first group was in quadrant 3, mainly PE and ether PE, which were higher in control samples. The second group contained the largest number of lipid species, corresponding to 100 °C-heated egg yolks. The third group was mainly TAG, corresponding to 200 °C-heated egg yolks, indicating that some TAGs had a higher content in fried egg yolks.

Change of different lipid subclasses for different treating temperatures

Different lipid subclasses, due to differences in molecular structure, tended to have different oxidation properties (Zhou et al. 2022a). Lipid molecular species data were classified and analyzed as shown in Fig. 4. The three egg yolk samples were distributed in different regions in the PLS-DA scores plot, indicating that the three samples could be clearly distinguished by lipid subclass. The scores plot showed that most lipids were higher in unheated egg yolks, while LPC, ether LPC and PI were higher in two heated egg yolks. The VIP scores of different lipid subclasses are shown in Fig. 4C. There are

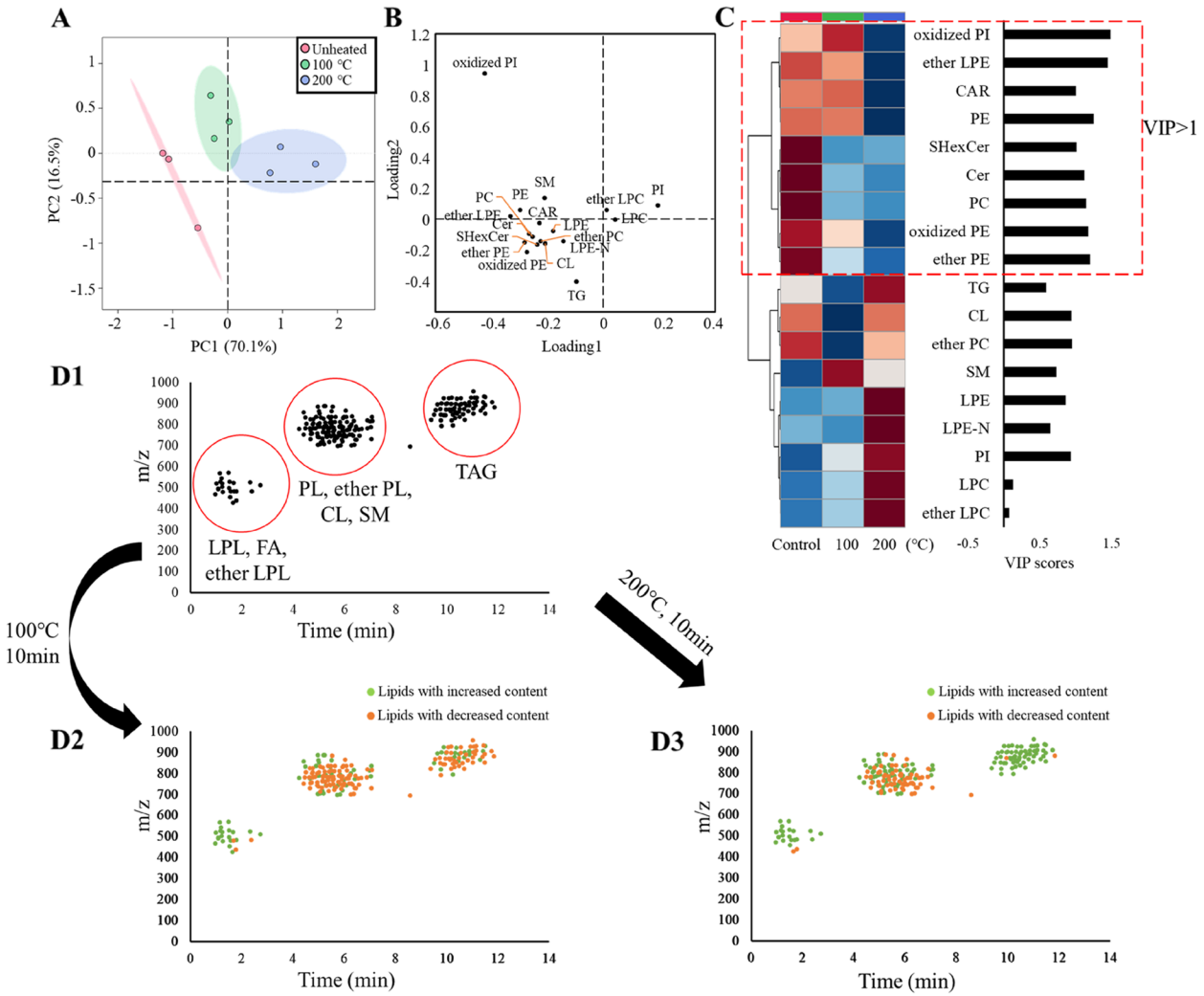


Fig. 4 Classification and analysis of lipids in quail egg yolks during thermal treatment. **A** Scores plot and **B** Loading plot based on PLS-DA; **C** Variable importance in projection (VIP) analysis of different types of lipids; **D** Scatter plot of retention time and m/z of lipid molecular species from **D1** Control samples, **D2** 100 °C-heated egg yolk samples and **D3** 200 °C-heated egg yolk samples. The fold calculation **D2** is the peak area of each lipid in the 100 °C-heated egg yolk samples divided by the peak area in the control samples, and **D3** is the peak area of each lipid in the 200 °C-heated egg yolk samples divided by the peak area in the control samples. The fold greater than 1 was marked in green and represented the lipid with increased content. The fold less than 1 was marked in orange and represented the lipid with decreased content. All assays were performed in triplicate

nine lipids with VIP greater than 1, and their contents all decreased during the heating treatment, indicating that the lipid oxidative degradation with the increase of heating temperature was the main trend in egg yolk. TAG and lysophospholipids (LPL) showed an increasing trend, but their VIP value was small, indicating that their content only increased by a small proportion. The latter was a product of phospholipids in the oxidation process, and the increase in their content was also in line with the oxidation rules (Zhou et al. 2022b). Therefore, phospholipids (PL), plasmalogens, etc. were the main flavor contributors during heating treatment. PE, ether PE and PC, as the four lipids with the high content, had larger VIP values, so they contributed more to the heat-processed flavor of quail egg yolk.

Figure 4D1 shows the distribution of all lipids, the orange dots in Fig. 4D2 and D3 represented lipids with reduced content during heating treatment, and green dots represented lipids with increased content. Compared to the 200 °C-heated egg yolks, more lipids in the 100 °C-heated egg yolks decreased, including phospholipids, glycerides, etc., but Fig. 1A showed that the 100 °C-heated egg yolks were closer to the raw egg yolks, indicating that the lipid change was smaller than that of the 200 °C-heated egg yolks. In the 200 °C-heated egg yolks, more phospholipids decreased than other lipids, which was consistent with the results of the VIP analysis. Most of the short-chain lipids, LPL, fatty acid (FA), and ether LPL, showed an upward trend at 100 and 200 °C, because they also played the role of downstream products in the normal oxidation process. Wang et al. found that some lysophospholipids in Antarctic krill oil would increase due to heating treatment (Chang et al. 2020). Our team's previous research also found that LPC, lysophosphatidylethanolamine (LPE) and FA in the lipid standard models would increase during heating treatment (Zhou et al. 2022a).

Analysis of the key flavor precursors

To clarify the flavor precursors in egg yolk, we conducted a correlation analysis between volatile compounds and lipids. 31 volatile compounds with VIP greater than 1 and 100 lipids most correlated with these volatiles were selected and analyzed, as shown in Fig. 5. Lipids that exhibited negative correlations with volatile compounds are considered potential precursors of these volatile compounds. Among the 17 key volatile compounds in fried egg yolks (Fig. 1E), 15 (including all pyrazines and aldehydes) showed negative correlations with LPE O-16:1, PE 18:1_18:1, PE 18:0_18:1, PE O-18:1_22:6, and PE 16:0_18:2. This indicated that the fried egg flavor might originate from PE-related lipids, especially the ones containing 18-carbon fatty acids. The significant changes of PE and ether PE in SAM analysis also proved that they played an important role in the flavor formation of fried egg yolk (Fig. S1A). Some studies have shown that lipid oxidation can affect the Maillard reaction (Zhao et al. 2022), and even the amino group of PE can directly participate in the Maillard reaction (Shrestha & De Meulenaer 2014; Zamora et al. 2011; Zhang et al. 2023). Therefore, PE may be involved in the formation of pyrazine in fried eggs. Numerous lipid species, including 27 TGs and 14 PCs, were found to exhibit negative correlations with the flavor compounds in boiled egg yolks, which may contribute to the boiled egg yolk flavor. This suggested that triglycerides and phosphatidylcholine (PC) may have a significant impact on the volatiles in boiled eggs, particularly triglycerides. The flavor effect of volatile substances in boiled eggs was far less than that of fried eggs, which could be related to lipid precursors. Chen et al. also observed that the addition of neutral lipids to chicken meat had minimal influence on their flavor (Chen et al. 2019). Therefore, a substantial portion of the volatile compounds in boiled eggs likely originated from neutral lipids, which could explain why boiled eggs possessed a comparatively weak flavor. The flavor precursors of the raw egg cannot be judged through the analysis in this study, because the source of its flavor is more complex, and may be related to the genetic composition (Gao et al. 2019), metabolism, growth environment and other factors of quail eggs.

Characterisation and variation of lipids oxidation products

To further explore lipid oxidation in egg yolks, oxidized lipids as oxidation products were focused on. The 10 important lipids with relatively high content and significant change during thermal treatment (Fig. S1B) were selected, and their corresponding oxidation products were manually calculated and identified. Finally, three oxidized PC and three oxidized PE were found (Fig. 6A). However, the lipid changes showed a decreasing trend, contrary to our initial expectations. Previous oxidation studies on pure oil systems have shown that most of the oxidized lipids tend to increase with heating treatments (Zhou et al. 2022b). After repeated checking of the mass spectra, the identification of oxidized lipids in this study was confirmed to be accurate. So it was speculated that these oxidized lipids were originally present in the yolk and declined along with normal phospholipids during thermal treatment. This may be due to the fact that only trace amounts of oxidized lipids were generated during the short heating period, which did not accumulate to detectable levels, whereas it is only the oxidized lipids present before the heating treatment were sufficient for detection by the instrument. To confirm this conclusion, the LC-MS data was further comprehensively analyzed.

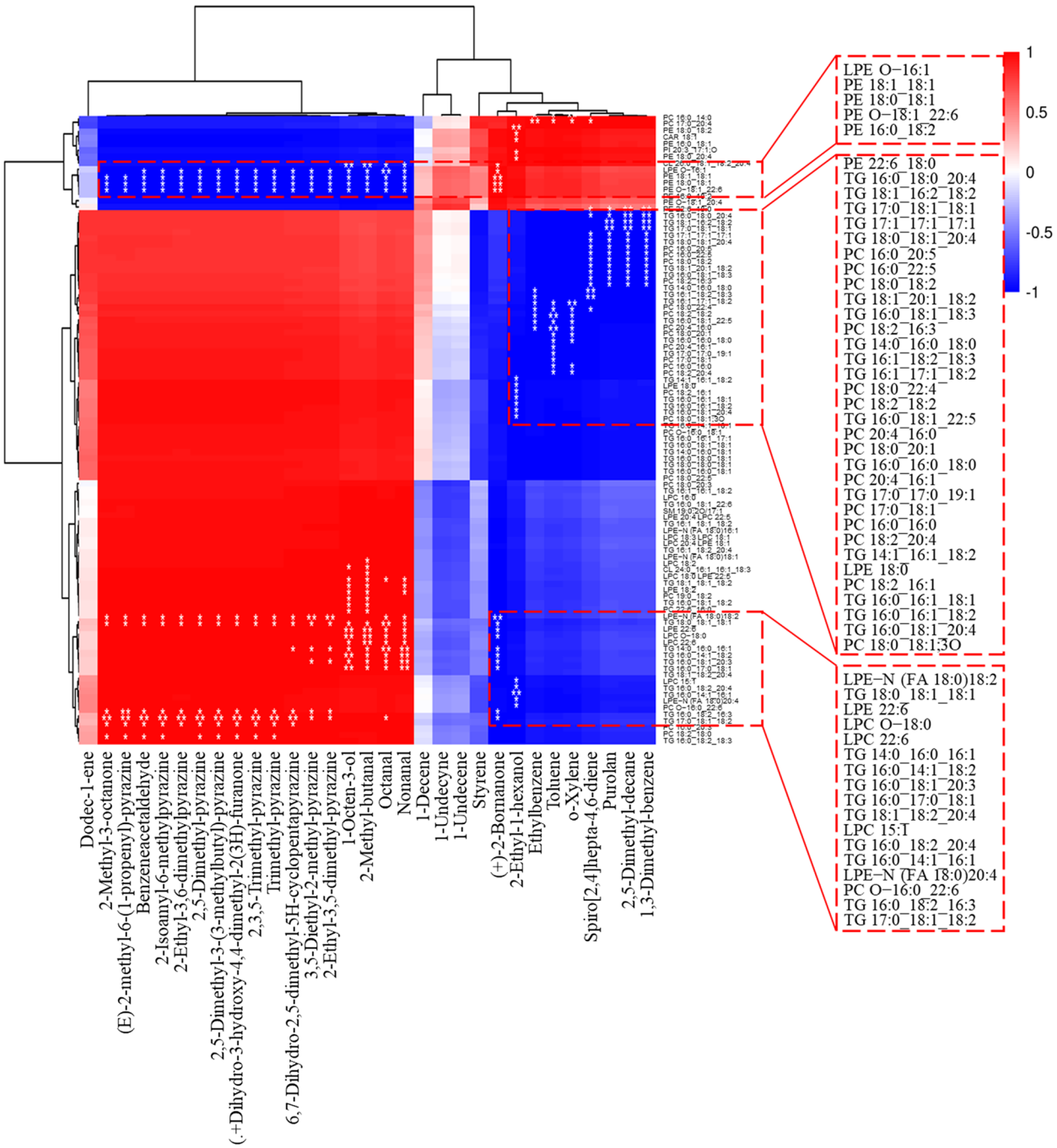


Fig. 5 Correlation between volatile compounds and lipid molecular species in quail egg yolks at different temperatures. All assays were performed in triplicate

A total of 1041 features with MS/MS were detected in positive and negative ion modes. As shown in Fig. 6B, each dot represented a feature, and a ratio greater than 1 indicated a content increase, and less than 1 indicated a content decrease. The sector chart showed the number of features with a ratio of 0.667-1.500 and features that were not in this range. Features in this range accounted for the majority, about 90% or more. This showed that there were fewer compounds with significant increases or decreases during thermal treatment. Although there is little overall change in lipid content, the few lipids that had a significant change may have a great influence

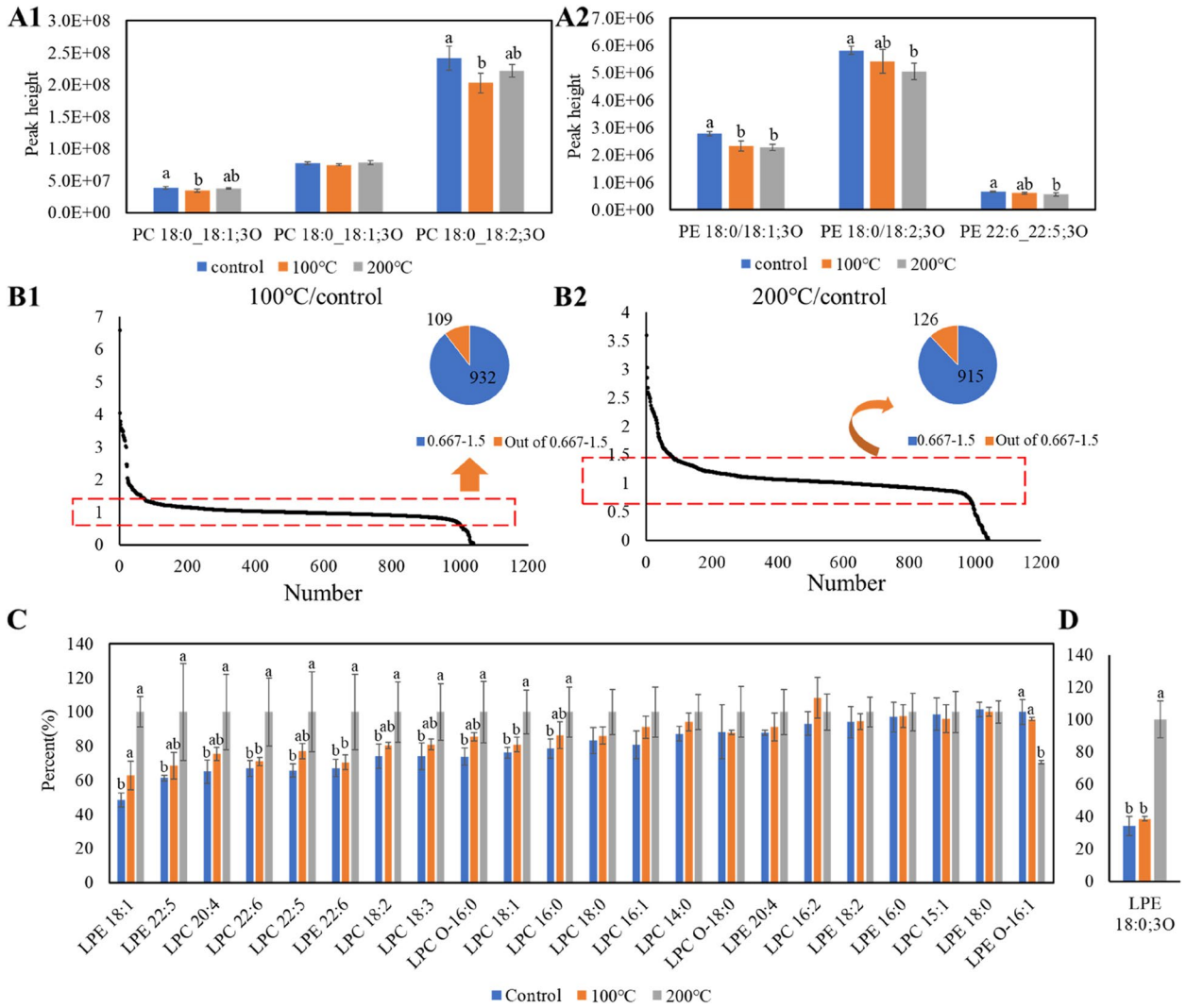


Fig. 6 Lipid oxidation products analysis during thermal treatment. **A** The change of oxidized lipids content; **B1** Scatter plot of feature change multiples (100 °C/control); **B2** Scatter plot of feature change multiples (200 °C/control); **C** The change of lysophospholipids; **D** The change of LPE 18:0;3O. The fold calculation **B1** is the peak area of each feature assigned with MS/MS in the 100 °C-heated egg yolk samples divided by the peak area in the control samples, and **B2** is the peak area of each feature assigned with MS/MS in the 200 °C-heated egg yolk samples divided by the peak area in the control samples. The number of features in the fold range from 0.677-1.5 and not in this range is presented as a sector graph. All assays were performed in triplicate

on egg yolk flavor. The absence of severe lipid oxidation in the egg yolk may be due to the short heating time. It may be also related to the higher phospholipid content in egg yolk, which has greater oxidation stability (Cui et al. 2015). In addition, most of the decreased oxidized lipids contained 18-carbon fatty acids. These lipids were associated with the formation of volatile compounds with 8–10 carbon in the egg yolk. In our previous research, it was found that oleic acid was very easy to form 8–10 carbon volatile compounds (Zhou et al. 2022a). Octanal, nonanal and (+)-2-Bornanone were volatile compounds with 8–9 carbon, and contributed a lot to fried quail egg yolk flavor, which may be formed from these phospholipids containing the 18-carbon fatty acids. Wang et al. explored the fatty acid composition in quail egg yolks and found that FA 18:1 had the highest content among fatty acids in quail eggs, and the 18-carbon fatty acids accounted for more than 60% of the total fatty acid content (Wang et al. 2014). Therefore, phospholipids containing the 18-carbon fatty acids may have an important

effect on the flavor of heat-treated quail egg yolks, especially for fried egg yolks. This is consistent with the result of the previous section, where it was also proved that the formation of volatile flavor compounds in fried egg yolks was related to PE-related lipids with 18-carbon fatty acids (Fig. 5). Lysophospholipid, as another important oxidation product of phospholipids, was detected as shown in Fig. 6C. LPC, ether LPC and LPE mostly showed an upward trend, while ether LPE showed a downward trend. It is possibly due to the poorer oxidation stability of plasmalogen, and ether LPE further generated other oxidation products. In addition, LPE 18:0;3O, an oxidation product of LPE or PE, was also found, which showed a significant upward trend during 200 °C thermal treatment. This also suggested that lipids containing 18-carbon fatty acids played an important role in the formation of the flavor of fried egg yolks.

Conclusion

In conclusion, the comprehensive lipidomics and flavoromics analysis revealed flavor profiles, key flavor compounds and flavor precursors in common quail egg yolk products. The raw and fresh flavor of raw egg yolks came from 1-Octen-3-ol, 2-Ethyl-1-Hexanol, 1-Decene, and 1-Undecene. 2-Methyl-3-octanone, Toluene, and some aromatic compounds worked synergistically to contribute to the aroma profile of boiled eggs. (+)-2-Bornanone,Octanal,2-Methyl-butanal,Nonanal,(.+Dihydro-3-hydroxy-4,4-dimethyl-2(3 H)-furanone, 6,7-Dihydro-2,5-dimethyl-5 H-cyclopentapyrazine, (E)-2-methyl-6-(1-propenyl)-pyrazine, 2-Ethyl-3,5-dimethyl-pyrazine, 3,5-Diethyl-2-methyl-pyrazine, 2,5-Dimethyl-pyrazine were the key flavor compounds in the fried egg and gave it the popcorn and roasted flavors. The typical boiled and fried method had little impact on the lipid nutritional value of quail egg yolks. Specifically, only a minor portion of lipids in egg yolk underwent significant changes after heating, which were primarily attributed to aroma formation. Notably, the PE-related lipids containing 18-carbon fatty acids played a crucial role in fried quail egg flavor formation, acting as flavor precursors of some important volatile compounds such as octanal, nonanal. The discovery of the key flavor compounds and their lipid precursors can provide a theoretical foundation for the synthesis of quail egg flavor essence and the flavor regulation of quail egg products.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-024-00234-8.

Additional file 1.

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Authors' contributions
Zheng Zhou: Investigation, Formal analysis, Resources, Validation, Writing-original draft; Haonan Jiang: Investigation, Formal analysis; Haoyuan Sun: Investigation, Formal analysis; Xu-Hui Huang: Writing-review & editing; Shuang Cui: Investigation, Formal analysis; Dayong Zhou: Conceptualization, Resources; Lei Qin: Conceptualization, Methodology, Resources, Funding acquisition, Writing-review & editing. All authors read and approved the manuscript.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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