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Quantitative lipidomics analysis of changes in egg yolk lipids during spray-drying and subsequent accelerated storage

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ABSTRACT

Egg yolks are rich in lipids that are easily altered during processing and storage. In this study, a liquid chromatography-tandem mass spectrometry strategy was used for quantitative lipidomics analysis of egg yolk after spray-drying processing and accelerated storage. Spray-drying treatment caused lipid oxidation (especially the oxidation of free fatty acids), potential hydrolysis of phospholipids, and alteration of the form of certain polyunsaturated fatty acids (docosahexaenoic acid, eicosapentaenoic acid, linolenic acid, and eicosatetraenoic acid) in egg yolk. These lipid alterations caused by the spray-drying process were further aggravated by the accelerated storage process. In detail, following storage, the abundance of free fatty acids, phosphatidic acid and phosphatidylethanolamine decreased further; and the abundance of polyunsaturated fatty acids in the form of triglycerides increased significantly. These results provide new insight into the mechanism underlying egg yolk property changes during spray-drying and storage, and offer valuable reference data for egg yolk powder promotion and application in food processing.

1. Introduction

Egg yolk, characterised by rich nutritional properties, is one of the most widely consumed foods on a daily basis, and is also an important ingredient in food processing (Xiao et al., 2020a). Egg yolk has many excellent functional properties such as coagulation, emulsification, and colouring, which further enhance the stability, nutritional value, and sensory quality of processed foods such as mayonnaise, ice cream, and cake (Laca et al., 2014). In addition, the components in egg yolk have broad application prospects, including for nutrient encapsulation and delivery (Ye et al., 2022) and in the preparation of functional active peptides (Zhao et al., 2021). As a result, egg yolk not only represents a common source of daily nutrition for consumers but is also widely used as a food supplement. However, the preservation of fresh egg yolk liquid is challenging and associated with high transportation costs. Processed egg yolk powder has the advantages of a long shelf life; high stability; and easy production, transportation, and storage (Li et al., 2022). Therefore, egg yolk powder is a preferred and more common choice in food processing at the industrial scale.

In industrial production, fresh egg yolk is mainly processed into egg yolk powder using spray-drying (Wenzel et al., 2010). Increased research attention has recently focused on the effects of different spray-drying process parameters on the quality of egg powder products in terms of moisture content, colour, odour, water solubility, and emulsification (Grassi and Ponsano, 2015; Rannou et al., 2013; Ulrichs et al., 2015). Recent years have also witnessed a trend of increased awareness of nutritional quality in the general public's consumption preferences and dietary attitudes. Therefore, determining the changes in yolk nutritional quality that occur during spray-drying processing and subsequent storage has become a new focus of research attention. For example, the nutrient content and fatty acid composition of egg yolks, egg whites, and whole eggs were reported to change after freezing and spray-drying treatments (akpınar et al., 2010). In contrast, Grassi et al. (Grassi et al., 2015) found that the temperature of spray-drying had a minimal effect on the overall levels of protein, lipids, and ash in egg yolk. Therefore, further research is needed to clarify the specific changes in the nutritional composition of egg yolk occurring during spray-drying and subsequent storage.

As the richest nutritional component of the egg, the egg yolk contains 30%–32.5% lipids, including a variety of sterolipids, fatty acyl esters, sphingolipids, isopentenolipids, glycerolipids, and glycerophospholipids

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Abbreviations

AOCS	American Oil Chemists' Society
ARA	eicosatetraenoic acid
BA	bile acid
CAR	acylcarnitine;
CDA	conjugated dienoic acid
Cer	ceramide;
CV	coefficient of variation
DAL	differentially abundant lipid
DG	diglyceride;
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FEY	freeze-dried egg yolk powder
FFA	free fatty acid
LA	linolenic acid
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LPC	lysophosphatidylcholine;
LPA	lysobisphosphatidic acid
LPE	lysophosphatidylethanolamine;
LPG	lysophosphatidylglycerol
LPI	lysophosphatidylinositol
OPLS-DA	orthogonal partial least-squares discriminant analysis
PA	phosphatidic acid
p-AV	p-anisidine value
PC	phosphatidylcholine;
PCA	principal component analysis
PE	phosphatidylethanolamine;
PG	phosphatidylglycerol
PI	phosphatidylinositol
PS	phosphatidylserine;
PUFA	polyunsaturated fatty acid
QC	quality control
SEY	spray-dried egg yolk powder
S-SEY	stored spray-dried egg yolk powder
SPH	sphingosine;
TG	triglyceride;
TIC	total ion current
UV	ultraviolet

(He et al., 2023). Many studies have shown that changes in yolk lipids during processing and storage are key contributors to the overall quality of egg yolk-derived products (Liu et al., 2023). Although some potential adverse changes in egg yolk lipids, such as a decrease in the content of some polyunsaturated fatty acids (PUFAs: eicosapentaenoic acid, docosahexaenoic acid, alpha-linolenic acid), have been investigated during spray-drying processing and subsequent storage (Javed et al., 2018), more in-depth studies are needed considering the high diversity of lipid species in egg yolk. With technological advances, quantitative lipidomics techniques have become powerful tools for studying the changes in various types of lipids during food storage and processing. Recently, Liu et al. (Liu et al., 2023) conducted a comprehensive lipidomic analysis of egg yolk at different storage times based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology, and found that the abundance of 159 lipid species changed significantly during storage (22 °C, 28 d), with phosphatidic acids, lysophosphatidic acids, and diglycerides identified as the most representative differentially abundant lipids (DALs) after storage. Meng et al. (Meng et al., 2022) analysed the changes in egg yolk lipids during storage using ultra-high-performance liquid chromatography-quadrupole-electrostatic field Orbitrap tandem mass spectrometry-based untargeted lipidomics, and found significant changes in the abundance of some triglycerides (TGs), phospholipids, and sphingolipids in egg yolk during high-temperature storage (30 °C, 10 d). Another study investigated the changes in lipid species in boiled egg yolk at different heat treatment intensities using LC-MS/MS-based lipidomics techniques, and found that some phospholipids were hydrolysed at certain heat treatment intensities. Thus, a preliminary understanding of the changes in fresh egg yolk lipids during processing and storage is currently available; however, a systematic understanding of the changes in lipid species during spray-drying and the subsequent storage of industrially produced egg yolk powder is still lacking. This also affects the further promotion and application of egg yolk powder products in food processing.

To fill this gap, in this study, we performed a preliminary investigation on the lipid stability of egg yolk during spray-drying processing and subsequent accelerated storage using quantitative lipidomics methods with the vacuum freeze-drying processing method as a control. This study can thus provide reference data for avoiding lipid nutrient loss and reduction of processing characteristics during the industrial processing of egg yolk.

2. Materials and methods

2.1. Egg yolk powder sample preparation

Fresh eggs (used within 72 h of laying, 62.0 ± 2.0 g) were purchased from the egg farm of Sichuan Sundaily Village Ecological Food Co. Ltd. (Mianyang, Sichuan). Fresh eggs from the same batch were randomly selected, the eggs were broken manually, the yolks were separated from the whites and rolled on filter paper to remove excess egg white and lacing, then the yolk membrane was punctured until a sufficient sample of yolk was collected. And the fresh sample of yolk liquid was prepared by magnetic stirring at 4 °C for 30 min (He et al., 2023). The fresh egg yolk liquid was vacuum freeze-dried with a cold trap temperature of −80 °C for 36 h (LC-12N–80C, Lichen Bonsi Instrument Technology Co., Ltd., Shanghai, China) to obtain freeze-dried egg yolk powder (FEY), which was used as a control. Spray-dried egg yolk powder (SEY) was obtained by referring to previous research methods with modifications for improvement (akpınar et al., 2010). The specific parameters set for the spray-dryer (BILON-6000Y, Bilang Instrument Manufacturing Co., Ltd., Shanghai, China) were: inlet air temperature of 180–185 °C, outlet air temperature of 80 °C or more, peristaltic pump feed volume of 500 mL/h, through-needle frequency of 15 s, inlet temperature of 28 °C, and fan speed of 4.6 m^3/min. The moisture content of the final collected egg yolk powder meets the product industry standard (≤4%).

High-temperature accelerated storage was used to simulate the industrial storage procedure and to compare the stability of egg yolk powder after spray-drying. The accelerated storage was carried out according to previous studies (Merkx et al., 2021; Tura et al., 2022). The SEY was stored at 60 °C for 3 days to obtain the stored SEY sample (S-SEY).

2.2. Lipid extraction

The three groups of samples (FEY, SEY, S-SEY) were selected at random and accurately weighed to 20 mg in a fresh centrifuge tube to which 1 mL of a mixture of methyl tert-butyl ether and methanol (3:1, v/v) containing an internal standard was added with steel balls. The tubes were shaken well with a ball mill (MM400, Retsch, Germany) for homogenisation. The beads were then removed, and the tubes were shaken for 2 min using a multi-tube vortex shaker (MIX-200, Jingxin Business Development Co., Ltd., Shanghai, China) and treated with ultrasound (KQ5200E, Kunshan Shumei Ultrasonic Instruments Co., Ltd., Kunshan,

China) for 5 min. An additional 200 μL of water was added and the tubes were vortexed for 1 min. After centrifugation at 15,160×*g* for 10 min at 4 °C (5424R, Eppendorf, Germany), 200 μL of the supernatant was aspirated into a fresh centrifuge tube and the solvent was evaporated using a vacuum concentrator (7310038, Labconco, Kansas City, MO, USA). Finally, the samples were dissolved again with 200 μL of acetonitrile solution containing 0.04% acetic acid for subsequent LC-MS/MS analysis. Three replicates were established for each group of samples (SEY, S-SEY, and FEY).

2.3. Quality control (QC) of liposome analysis

Equal quantities of samples from the three treatment groups were mixed and used as QC samples for reproducibility and accuracy analyses (Liu et al., 2023). During the instrumental analysis, a QC sample was inserted for every 10 assays of the analysis samples and the total ion current (TIC) of the MS assay of the same QC sample was analysed in an overlapping presentation. Pearson correlation analysis was also carried out on the QC samples. Finally, the empirical cumulative distribution function was used to analyse the frequency of material coefficients of variation (CV) below the reference value.

2.4. LC-MS/MS

We used an ExionLC AD UPLC system for LC, followed by the QTRAP 6500+ system for MS/MS (https://sciex.com.cn/). Chromatographic separation was performed with a Thermo Accucore C30 column (2.6 μm, 2.1 mm × 100 mm internal diameter). Mobile phase A was acetonitrile: water (60:40, v/v) containing 0.1% formic acid (10 mmol/L ammonium formate) and mobile phase B was acetonitrile:isopropanol (10:90, v/v) containing 0.1% formic acid (10 mmol/L ammonium formate). The gradient elution program was set to 80:20 (v/v) A:B at 0 min, 70:30 (v/v) A:B at 2 min, 40:60 (v/v) A:B at 4 min, 15:85 (v/v) A:B at 9 min, 10:90 (v/v) A:B at 14 min, 5:95 (v/v) A:B at 15.5 min, 5:95 (v/v) A:B at 17.3 min, 80:20 (v/v) A:B at 17.5 min, and 80:20 (v/v) A:B at 20 min. The injection volume was 2 μL, the flow rate was set to 0.35 mL/min, and the column temperature was 45 °C.

MS was performed with an electrospray ionisation source at 500 °C. The MS voltage was 5500 V in positive-ion mode and −4500 V in negative-ion mode; ion source gas 1 and gas 2 were set to 45 psi and 55 psi, respectively, and the curtain gas was set to 35 psi. In triple-quadrupole mode, each ion pair was scanned for detection according to the optimised declustering potential and collision energy.

2.5. Lipid characterisation and quantification

Firstly, the lipids of the samples were characterised by MS based on the retention time and parent-child ion pair information of the test substances, combined with information from the self-built Metware Database (more than 3000 lipid molecules, Metware Biotechnology Co., Ltd. Wuhan, China). And the CAS number, KEGG pathway, and other supporting annotation information were determined, thus improving the accuracy of the qualitative analysis of the substance. Lipid quantification was performed in multiple reaction monitoring mode using triple-quadrupole MS data (Liu et al., 2023). Analyst 1.6.3 was used to process the MS data. The peaks detected for each substance in the different samples were first corrected to ensure accurate quantification. The actual abundance of the substance in the sample was then calculated from the area ratio of the peak areas of all samples detected according to the internal standard method (Table S1) with the following formula:

$$X = 0.001 \times R \times c \times F \times V/m$$

where X is the amount of lipid in the sample (nmol/g), R is the ratio of the peak area of the substance to be measured to that of the internal standard, F is the correction factor for internal standards of different types of substances, c is the concentration of the internal standard (μmol/L), V is the volume of the sample extraction solution (μL), and m is the mass of the sample (g).

2.6. Determination of lipid oxidation indicators

The egg yolk oil was prepared according to an improvement of a published protocol (Bligh and Dyer, 1959). In brief, 5 g of egg yolk powder (FEY and SEY samples with storage at 60 °C for 3 days and without storage treatment) was accurately weighed, added to 30 mL of chloroform:methanol (2:1, v/v), and mixed thoroughly. Extraction was carried out at 20 °C for 1 h with a magnetic stirrer, followed by filtration. Three times the volume of 7% NaCl solution was added to the obtained filtrate to promote partitioning. The lower layer was obtained using a separatory funnel and excess chloroform solution was removed using a nitrogen blowing apparatus (MD200-1, Aosheng Instruments Co., Ltd., Hangzhou, China). The resulting egg yolk oil was placed in brown bottle and stored at 0 °C until analysis.

Analysis of the primary oxidation product conjugated dienoic acid (CDA) was performed according to the American Oil Chemists' Society (AOCS) method (Brühl, 1997). In brief, 100 mg of egg yolk oil was accurately weighed and dissolved in 25 mL of isooctane, which was left to stand for 10 min in the dark. The mixture was diluted 10 times with isooctane and the absorbance value at 233 nm (A_{233}) was measured using an ultraviolet (UV) spectrophotometer (UV1901PC, Aoan Scientific Instruments Co., Ltd., Shanghai, China), with three replicate measures taken. The relative abundance (%) of CDA in the sample was calculated according to the following formula:

$$\text{CDA}\ (\%) = (0.84 \times A_{233})/(b \times c/K)$$

where A_{233} is the absorbance value of the egg yolk oil at 233 nm, b is the optical diameter of the cuvette (1 cm), c is the concentration of the solution to be measured (g/L), and K is the absorbance coefficient of acids (0.03).

Changes in the *p*-anisidine value (*p*-AV) reflect changes in secondary products such as aldehydes, ketones, and quinones during the oxidation of oils and fats, which is widely used as a measure of the degree of secondary oxidation of oils and fats. Referring to the AOCS method (Brühl, 1997), 100 mg of egg yolk oil was accurately weighed, dissolved in 25 mL of isooctane, and the absorbance value (A_1) was measured at 350 nm using the UV spectrophotometer (UV1901PC, Aoan Scientific Instruments Co., Ltd.). Subsequently, 0.5 mL of 0.5% (w/v) anisidine solution dissolved in acetic acid was added to the 2.5 mL of egg yolk oil dissolved in isooctane, left for 10 min, and the absorbance value (A_2) was measured at 350 nm with three repeated measures. The *p*-AV was then calculated using the following formula:

$$p\text{-AV} = 25 \times [1.2 \times (A_2 - A_1)]/W$$

where A_1 is the absorbance value of the mixture at 350 nm before the addition of anisidine solution, A_2 is absorbance value of the mixture at 350 nm after the addition of anisidine solution, and W is the mass of the egg yolk oil sample (g).

2.7. Data analysis

All tests in this study were repeated three times. Data are expressed as mean ± standard deviation. One-way analysis of variance was performed using GraphPad Prism 8.0 software, with $p < 0.05$ indicating significant differences (Wang et al., 2021). Multivariate statistical analyses, including principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA), were further carried out (Thévenot et al., 2015; Wang et al., 2022). Unsupervised PCA was performed by the statistical function *prcomp* in R software (www.r-project.org). The data were unit variance-scaled before unsupervised PCA. The OPLS-DA was performed using the MetaboAnalystR package

OPLSR. Anal function in R software.

3. Results

3.1. Data quality evaluation of lipidome analysis

The stability of the instrument used during the testing was evaluated by analysing the TIC plot of the MS detection analysis of the QC samples. The curves of the total ion flow for lipid detection were highly overlapping (i.e., the retention times and peak intensities were consistent), indicating good signal stability when MS detected the same sample at different times (Fig. 1A and B). In addition, the Pearson correlation coefficients of QC samples were greater than 0.99, indicating good stability of the whole detection process (Fig. 1C). Moreover, the CV, as the ratio of the standard deviation of the original data to the mean of the original data, can reflect the degree of dispersion of the data to some extent. In this study, the percentage of QC samples with CV values less than 0.3 was higher than 85% (Fig. 1D), again indicating that the experimental data were very stable. This high stability of the instrumentation provided an important guarantee of the reproducibility and

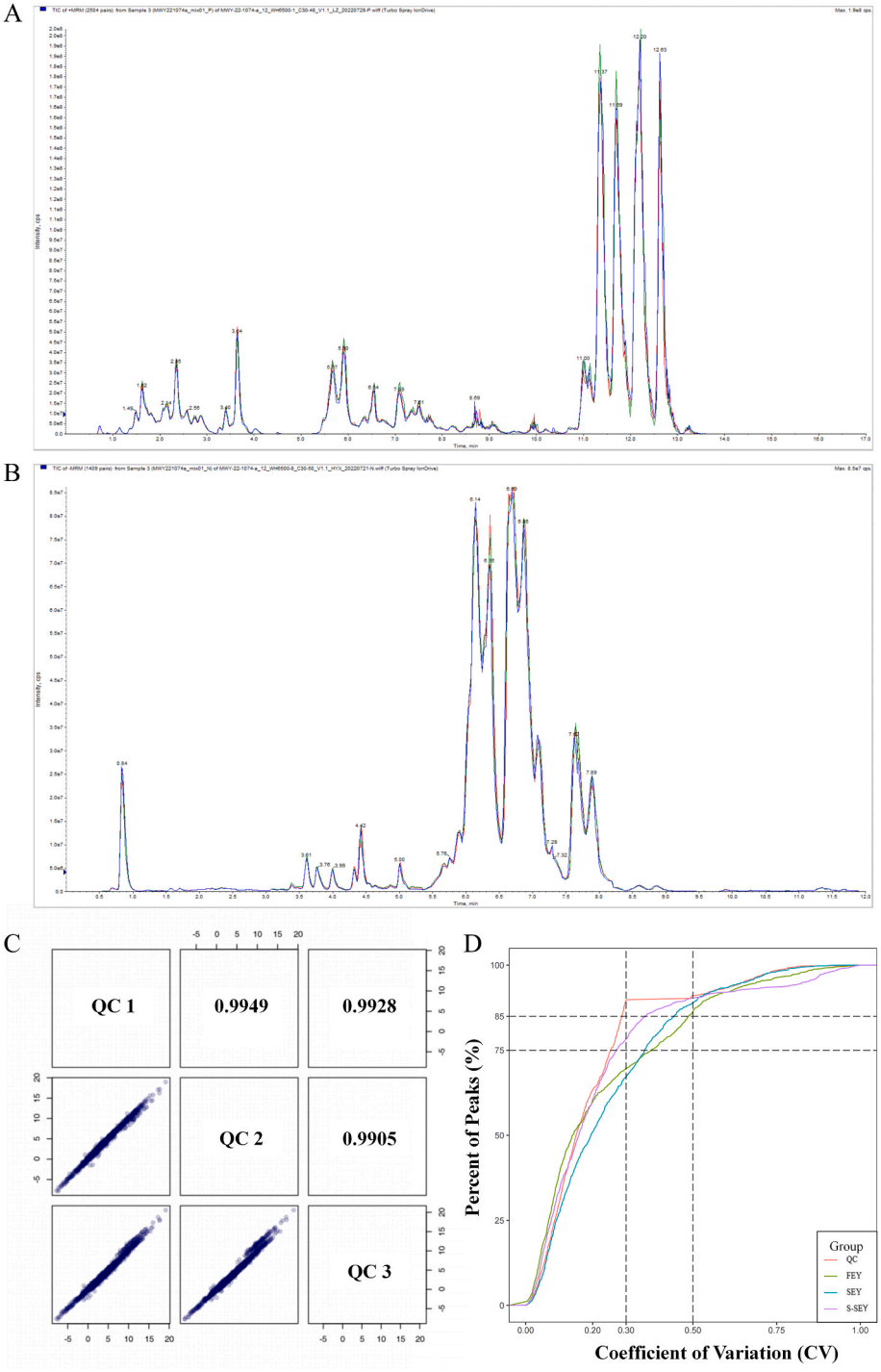

Fig. 1. Results of quality control (QC) sample analysis in the egg yolk lipidomic assay. (A) Total ions current (TIC) plot for mass spectrometry analysis of the QC sample of the same mass in positive-ion mode. **(B)** TIC plot for mass spectrometry analysis of the QC sample of the same mass in negative-ion mode. **(C)** Pearson correlation analysis of QC samples. **(D)** Distribution of coefficient of variation (CV) values of QC samples.

reliability of the data obtained from the lipidomics assay used in this study.

3.2. Overview of the lipidomic results of egg yolk

A total of 1194 lipid species were detected in the three groups of samples: FEY, SEY, and S-SEY (Table S2). These lipid species belonged to six major lipid categories (sterolipids, fatty acyl esters, sphingolipids, isopentenolipids, glycerolipids, and glycerophospholipids), with 24 lipid subcategories, dominated by 230 TGs, 182 phosphatidylethanolamines (PEs), 159 ceramides (Cers), 124 phosphatidylcholines (PCs), 72 phosphatidylglycerols (PGs), 70 phosphatidylserines (PSs), 51 lysophosphatidylcholines (LPCs), 48 lysophosphatidylethanolamines (LPEs), 42 phosphatidylinositols (PIs), and 37 acylcarnitines (CARs) (Fig. 2A). Among them, the following 13 lipid species were not detected in the FEY control samples: carnitine C10:2, oxidized lipid_11(S)-HETE (11(S)-HETE), oxidized lipid_9,10-EpOME (9,10-EpOME), oxidized lipid_12,13-EpOME (12,13-EpOME), lysobisphosphatidic acid (LPA) (22:6), PC (20:4_20:5), PG (21:0_20:4), PI (20:4_20:1), PI (18:1_20:1), PS (18:2_18:1), PS (16:0_18:1), PS (20:0_20:4), and PS (18:1_18:1).

3.3. Effects of spray-drying and accelerated storage on lipidomics of egg yolk

To investigate the lipid molecular species changes of egg yolk powder after spray-drying treatment and to further investigate the lipidomic characteristics of egg yolk powder during accelerated storage, multivariate statistical analysis was performed on the obtained lipidomic data of the three groups of egg yolk powder. First, to understand the overall lipid differences between groups and the magnitude of variability between samples within groups, PCA was performed on the samples, which showed a clear separation between the FEY, SEY and S-SEY groups (Fig. 2B). In addition, the three groups of samples showed the greatest separation in the PC1 dimension, with a 38.40% difference. This indicated that the lipids of the spray-dried egg yolk powder changed significantly compared with the vacuum freeze-dried egg yolk powder, and the lipids of the egg yolk powder obtained by spray-drying also changed somewhat during accelerated storage. Further group comparisons using OPLS-DA showed significant differences between the SEY and S-SEY groups (Fig. S1). These results further confirmed that spray-drying processing and accelerated storage processes result in significant changes in the lipidomic profiles of egg yolk.

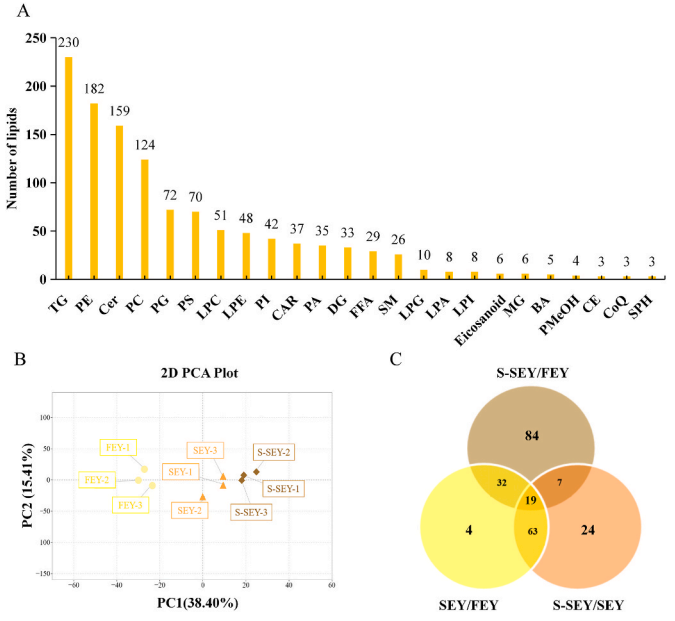

Fig. 2. Lipidomic analysis of egg yolk. (A) Number of lipids contained in the lipid species identified in FEY, SEY, and S-SEY groups. **(B)** Principal component analysis (PCA). **(C)** Venn diagram of differentially abundant lipids (DALs) distribution. FEY, freeze dried egg yolk powder; SEY, spray-dried egg yolk powder; S-SEY, accelerated stored spray-dried egg yolk powder.

3.4. Effects of spray-drying and accelerated storage on the total abundance of lipid classes in egg yolk

Changes in the total abundance of the 24 lipid subcategories in the three groups of egg yolk powder were compared. As shown in Fig. 3, the abundance of LPA and lysophosphatidylinositol (LPI) significantly increased in SEY compared to FEY, whereas the abundance of lysophosphatidylglycerol (LPG), sphingosine (SPH), CARs, and eicosanoid significantly decreased. Compared with FEY samples, S-SEY samples showed a more significant increase in the abundance of LPA, LPC, and LPI, whereas free fatty acids (FFAs), phosphatidic acid (PA), PE, LPG, SPH, CAR, bile acids (BAs), and coenzyme Q (CoQ) showed a more significant decrease in abundance.

3.5. Differentially abundant lipids (DALs) in the three groups of egg yolk

The variable importance in projection (VIP $\geq$1) based on the OPLS-DA model was investigated, along with the fold change (FC $\geq$ 2.0 or $\leq$ 0.5) values from univariate analysis to further screen out DALs. The data from the three yolk lipidomic profiles were compared, and a total of 233 DALs were obtained (Fig. 2C).

Analysis of the changes in the abundance of lipid species due to the spray-drying process revealed that 118 (99 increased, 19 decreased) DALs in the SEY vs. FEY comparison belonged to 13 lipid subcategories (Fig. S2A). These DALs included 64 Cers (increased), 10 PIs (2 increased, 8 decreased), 10 PSs (9 increased, 1 decreased), 5 PCs (4 increased, 1 decreased), 5 eicosanoids (3 increased, 2 decreased), 4 PEs (decreased), 4 PGs (increased), 4 LPAs (increased), 4 CARs (2 increased, 2 decreased), 3 LPEs (increased), 3 diglycerides (DGs; 2 increased, 1 decreased), 1 PA (increased), and 1 BA (increased).

Analysis of changes of lipid species in spray-dried egg yolk powder during accelerated storage revealed a total of 113 (16 increased, 97 decreased) DALs belonging to 12 lipid subcategories in the S-EY vs. SEY comparison (Fig. S2B). These included 82 Cers (decreased), 6 PSs (4 increased, 2 decreased), 5 eicosanoids (4 increased, 1 decreased), 4 CARs (decreased), 3 BAs (1 increased, 2 decreased), 3 LPAs (increased), 2 PCs (decreased), 2 PGs (1 increased, 1 decreased), 2 LPEs (2 increased), 2 DGs (decreased), 1 PI (decreased), and 1 LPI (increased).

Finally, analysis of changes in egg yolk lipid species after spray-drying processing and accelerated storage, with relevance for practical production applications, revealed 142 (52 increased, 90 decreased) DALs in the S-SEY vs. FEY comparison (Fig. S2C). These DALs belonged to 17 lipid subcategories, including 29 PIs (2 increased, 27 decreased), 20 PEs (1 increased, 19 decreased), 20 PSs (11 increased, 9 decreased), 10 FFAs (10 decreased), 9 PCs (4 increased, 5 decreased), 7 CARs (2 increased, 5 decreased), 7 LPAs (increased), 7 LPIs (increased), 6 eicosanoids (4 increased, 2 decreased), 6 PGs (4 increased, 2 decreased), 5 PAs (2 increased, 3 decreased), 4 Cers (decreased), 3 LPEs (2 increased, 1 decreased), 3 LPCs (increased), 3 DGs (1 increased, 2 decreased), 2 BAs (1 increased, 1 decreased), and 1 TG (increased).

4. Discussion

4.1. Lipid oxidation of egg yolk lipids during spray-drying and accelerated storage

Egg yolk is rich in lipids, which are prone to oxidation during processing and storage (Meynier et al., 2014), representing a concern for reducing the yolk's nutritional and organoleptic properties. Currently, egg yolk powder is mainly produced by spray-drying, and egg yolk lipids

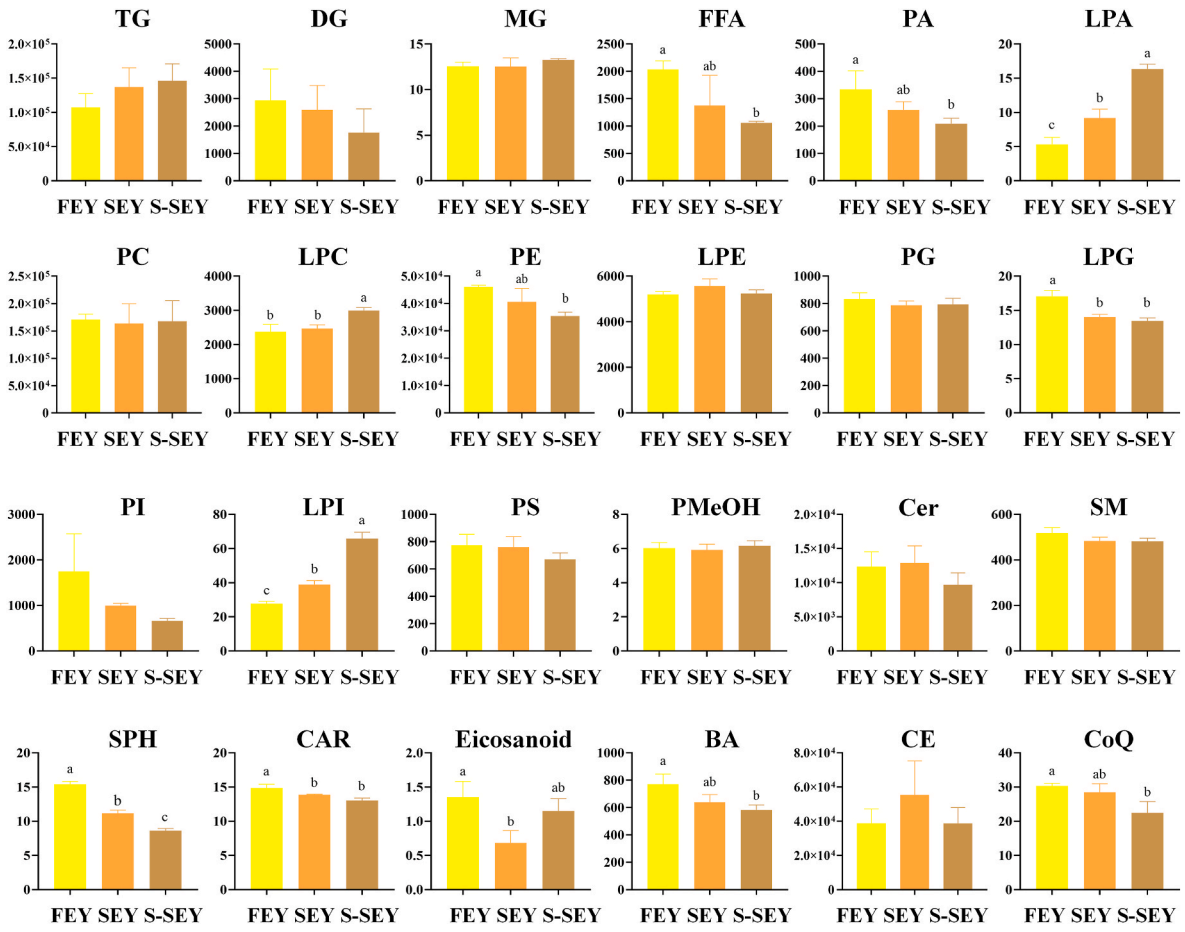

Fig. 3. Changes in the abundance of lipid categories in egg yolk during spray-drying processing and accelerated storage. FEY, freeze dried egg yolk powder; SEY, spray-dried egg yolk powder; S-SEY, stored spray-dried egg yolk powder. Significant differences ($p < 0.05$) between three groups are indicated by different lowercase letters (a–c).

must undergo a series of high-temperature treatments. The input port air temperature is typically set to 150–200 °C and the output port air temperature is generally below 100 °C. In addition, the atomization of spray-drying greatly increases the contact area of egg yolk with oxygen. These series of factors will likely accelerate the reaction between the egg yolk lipid molecules and oxygen (Meynier et al., 2014). However, there is a lack of reports on the systematic effects of spray drying processing and storage treatments on the oxidation of egg yolk lipids.

The present study further confirmed this speculation. The number of unsaturated double bonds in yolk lipids, especially phospholipids, is considered to be an important indicator of the antioxidant capacity and is also used as an important parameter to evaluate the nutritional value of yolk lipids (Bandarra et al., 2016; He et al., 2023). We found that the abundance of phospholipids containing two or more unsaturated double bonds in the SEY samples decreased from 124,222.72 to 114,261.30 nmol/g, representing an 8.02% decrease compared with that of the FEY samples (Fig. 4A). The decrease in the relative abundance of polyunsaturated phospholipids suggested that lipid oxidation occurs in SEY. This was further evidenced by the significant increase in conjugated dienoic acid (CDA)values in SEY samples (Fig. 4E). In addition, because FFAs are more susceptible to oxidation reactions (Erbay and Koca, 2019), the changes in FFAs during spray-drying were further investigated. Along with the decrease in the abundance of FFAs by 32.38% ($p > 0.05$) (Fig. 3), the abundance of free PUFAs also decreased from 797.83 nmol/g to 559.73 nmol/g ($p > 0.05$, Fig. 4B) in SEY compared with FEY samples. Javed et al. (2018) also found a significant decrease in the content of some PUFAs in egg yolk after spray-drying treatment. In addition, six oxidized lipids were identified in SEY. Among them, the abundance of 12,13-EpOME, 9,10-EpOME, oxidized lipid_(±)5-HETE ((±)5-HETE), and 11(S)-HETE significantly increased in SEY compared to FEY ($p < 0.05$), whereas oxidized lipid_PGE2 (PGE2) and oxidized lipid_TxB3 (TxB3) showed a significant decrease in SEY ($p < 0.05$) (Fig. 4D). A potential decrease in the abundance of the corresponding FFAs such as arachidonic acid (C20:4) and linoleic acid (C18:2) was also found (Fig. 4C). In addition, the metabolites of arachidonic acid or linoleic acid, such as 8-HETE (from arachidonic acid), 12,13-DHOME, 13-HODE, 9-HODE, 9,12,13-THOME (from linoleic acid), were reported to potentially increase the risk of ovarian cancer in women (Hada et al., 2019).

In the food industry, accelerated storage experiments are commonly used to simulate the assessment of the quality stability of processed products, and are mostly applied to the oxidative stability assessment of some oil and grease products (Mancebo-Campos et al., 2022; Merkx et al., 2021; Tura et al., 2022). As spray-drying is intended for long-term storage, we further focused on the effects of accelerated storage treatment following spray-drying on the lipid profiles of egg yolk. The yolk lipid species in the S-SEY group underwent a more severe oxidation reaction compared to those of the SEY treatment. The abundance of phospholipids containing two or more unsaturated double bonds in spray-dried egg yolk powder after accelerated storage was further reduced to 110,462.48 nmol/g, representing a 3.32% decrease compared to SEY (Fig. 4A). Similar to the results for the SEY treatment, the abundance of FFAs in the S-SEY group decreased by 47.88% ($p < 0.05$) and the abundance of PUFAs decreased further from 559.73 nmol/g in SEY to 431.98 nmol/g in S-SEY ($p < 0.05$, Fig. 4B). A similar trend but deeper change in corresponding FFAs and oxidized lipids

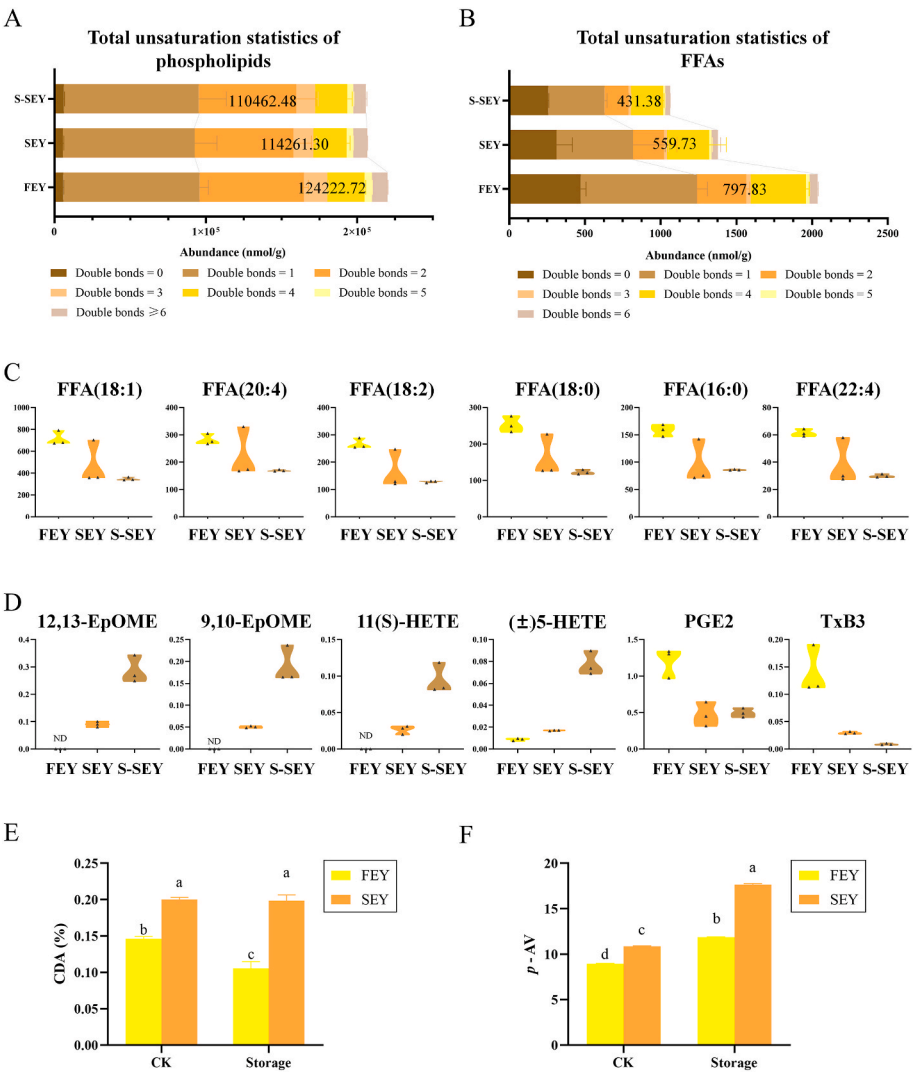

Fig. 4. Oxidation of egg yolk lipids during spray-drying processing and accelerated storage. (A) Variation in abundance values of phospholipids containing different numbers of unsaturated double bonds. **(B)** Variation in abundance values of free fatty acids (FFAs) containing different numbers of unsaturated double bonds. **(C)** Comparison of abundance values of the top six FFAs. **(D)** Changes in the abundance of six oxidized lipid species. FEY, freeze dried egg yolk powder; SEY, spray-dried egg yolk powder; S-SEY, stored spray-dried egg yolk powder. Significant differences ($p < 0.05$) between four groups are indicated by different lowercase letters (a–d).

occurred in S-SEY compared to the FEY and SEY groups (Fig. 4C and D). The intense lipid oxidation of fresh egg yolk during storage has been reported in previous studies and may be highly correlated with the action of endogenous lipase in egg yolk (Wang et al., 2017). The present study further demonstrated that even short high-temperature treatment induced by spray-drying will cause a certain degree of lipid oxidation of egg yolk powder during accelerated storage. On the one hand, it is possible that the heat treatment conditions of spray-drying did not completely inactivate some of the endogenous lipases in the egg yolk powder. For example, some studies have indicated that heat treatment during spray-dried milk powder production can enhance lipase activity (Kim et al., 2010), whereas other studies put forth the opposite view (Erbay et al., 2019). Alternatively, the high-temperature treatment conditions may also affect the structure of the protein-lipid complex in egg yolk, leading to accelerated spontaneous oxidation of egg yolk lipids (Innosa et al., 2019). For example, thermal energy can trigger the production of free radicals and thus potentially induce reactions in lipids (Cheng et al., 2017; Tang et al., 2021). Therefore, the mechanism of lipid oxidation during the accelerated storage of spray-dried egg yolk powder needs to be further investigated.

The changes in CDA values and anisidine values (*p*-AV) in SEY and S-SEY further validated the lipidomic findings. The values of CDA and *p*-AV were significantly higher in the SEY and S-SEY groups compared with those of the FEY samples ($p < 0.05$, Fig. 4E and F). The CDA value, as an important indicator of the oxidative decomposition of PUFAs into hydroperoxides, reflects the degree of primary lipid oxidation to some extent (Shahidi and Zhong, 2015). The *p*-AV mainly reflects the production of volatile small-molecule unsaturated aldehydes, which are secondary oxidation products of lipids. Lipid oxidation occurring during spray-drying processing and subsequent accelerated storage could potentially adversely affect the sensory evaluation and nutritional value assessment of egg yolk powder. For example, one study found that the odour intensity of spray-dried egg yolk powder increased during storage (Rannou et al., 2013). To effectively ensure the processing characteristics and nutritional value of spray-dried egg yolk products, it is necessary to minimize the loss of lipid oxidation during processing and storage, such as by the addition of some antioxidants. A previous study found that 150 mg/kg of heart-free acid, an antioxidant, was effective in preventing lipid oxidation and colour loss in egg yolks (Abreu et al., 2014).

4.2. *Potential hydrolysis of egg yolk phospholipids during spray-drying and accelerated storage*

Phospholipids are of great interest as an important component of egg yolk lipid nutrition. Given the significant changes identified in the abundance of some egg yolk phospholipid species during spray-drying processing and accelerated storage (Fig. 3), we further examined the abundance of the top six phospholipid species in the major phospholipid classes (PA, LPA, PC, LPC, PE, and LPE) in yolk lipids (Fig. S3).

The abundances of PA, PC, and PE in the top six abundances

decreased to some extent in the SEY compared with FEY samples. Gallier et al. (Gallier et al., 2010) also found the loss of phospholipid species such as PE in the spray-drying process to produce buttermilk powder. Tang et al. (Tang et al., 2021) also found that the concentration of phospholipids in milk, especially PE and PI, was severely influenced by the spray-drying process. In addition, significant increases in the abundance of lysophospholipid species such as LPA (18:1), LPA (20:4), LPC (0:0/16:0), LPC (22:6), LPE (18:0/0:0), LPE (0:0/16:0), and LPE (0:0/18:0) were found during the spray-drying treatment in the present study. Some studies have reported an increase in the abundance of some lysophospholipid species in spray-dried processed food fractions, but the corresponding mechanisms of occurrence remain unclear (Ali et al., 2018; Tang et al., 2021). Egg yolk is rich in endogenous lipases such as phospholipase and neutral lipase (Parchem and Bartoszek, 2016; Wang et al., 2017). During the spray-drying heating process, phospholipase activity may be enhanced, leading to phospholipid hydrolysis processes. Some studies have indicated that heat treatment during spray-drying can enhance the lipase activity in milk powder (Kim et al., 2010). The results of the SEY vs. FEY DALs screening also further suggest that the loss of some phospholipid species leads to an potential increase in the abundance of the corresponding lysophospholipid species (Fig. 5A). For example, in addition to LPA (18:1) and LPA (20:4), significant increases in the abundance of LPA (18:0), LPA (22:6), and LPE (22:0) were found. Correspondingly, the phosphatidic species PC (13:0_18:1), PE (22:1_18:0), PE (18:0_22:1), PE (O-20:0_22:4), PE (O-20:1_22:6), PI (17:0_18:0), PI (18:0_13:1), PI (18:0_14:1), and PS (20:4_20:4) showed significantly reduced abundance in the SEY compared with FEY samples.

The phospholipids in the SEY underwent more pronounced changes during subsequent accelerated storage. Among them, a more significant decrease in the abundance of PA and PE occurred in the top six abundances in S-SEY compared to SEY ($p < 0.05$) (Fig. S3). Accordingly, further significant increases in the abundance of most of the top six LPA, LPC and LPE were found. Therefore, further hydrolysis of some high-abundance phospholipids may have occurred during the subsequent accelerated storage of spray-dried egg yolk powder. In previous studies, it has been found that egg yolk phospholipids undergo violent metabolic reactions during storage, leading to a decrease in the level of phospholipids (Liu et al., 2023; Wang et al., 2017). The S-SEY vs. SEY DALs screening further indicated that spray-dried egg yolk lipids showed a loss of some phospholipids and an increase in the abundance of some

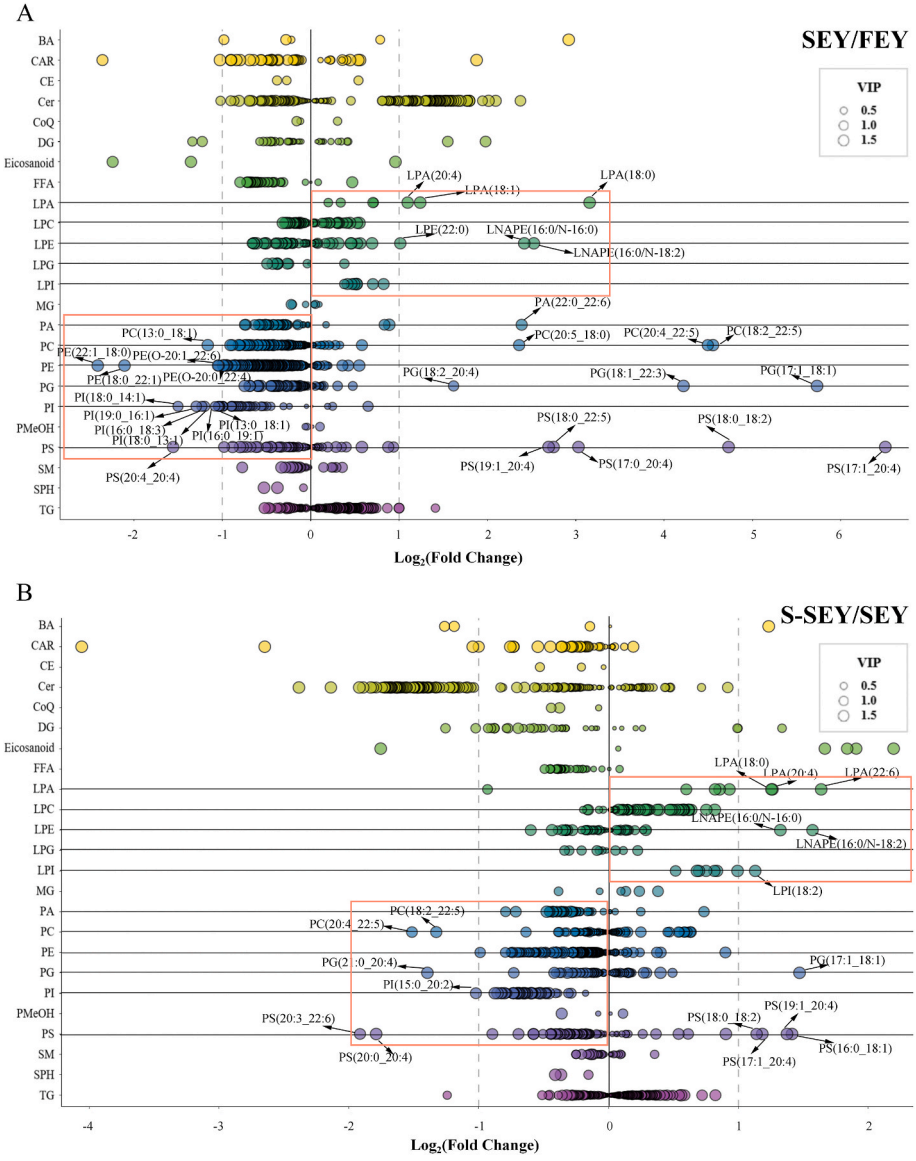

Fig. 5. Scatter plot of the distribution of differentially abundant phospholipids in egg yolk during spray-drying processing and accelerated storage. (A) SEY vs. FEY; **(B)** S-SEY vs. SEY. FEY, freeze dried egg yolk powder; SEY, spray-dried egg yolk powder; S-SEY, accelerated stored spray-dried egg yolk powder.

corresponding lysophospholipids during accelerated storage (Fig. 5B). Namely, increases in the abundance of LPA (18:0), LPA (20:4), LPA (22:6), and LPI (18:2) were found, accompanied by decreases in the abundances of PC (18:2_22:5), PC (20:4_22:5), PG (21:0_20:4), PI (15:0_20:2), PS (20:3_22:6), and PS(20:0_20:4).

As a special phospholipid molecule, sphingolipids contain many biologically active metabolic components such as Cer, sphingomyelin, and sphingosine phosphate, which may have potential benefits for human health (Rombaut and Dewettinck, 2006). The total abundance of SM and Cer in egg yolk did not change significantly ($p > 0.05$) during processing and accelerated storage. However, a significant increase in the abundance of 64 Cers during spray drying processing (Fig. S4) and a significant decrease in the abundance of 82 Cers during subsequent accelerated storage, resulting in a significant decrease in the abundance of only four Cers in the final S-SEY relative to FEY comparison. The overall increase in the Cers abundance detected during the spray-drying process in this study may be due to the metabolism of glycerophospholipids such as hydrolysis; however, the specific mechanism of the reaction and the decrease in abundance during the accelerated storage process need to be further investigated.

4.3. Changes in the form of PUFAs in egg yolk during spray-drying and accelerated storage

As mentioned above, in the analysis of changes in FFAs and phospholipids, some lipid species containing PUFAs [e.g., FFA (20:4), FFA (22:6)] were found to undergo significant changes during spray-drying processing and accelerated storage of egg yolk powder. Some PUFAs in egg yolk [such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), linolenic acid (LA, C18:3), and eicosatetraenoic acid (ARA)] are widely regarded for their high nutritional value (Bridges et al., 2020; Xiao et al., 2020b). A total of 76 lipids containing DHA, 21 lipids containing EPA, 53 lipids containing LA, and 100 lipids containing ARA were identified (Table S3). In contrast, only 54 lipids containing DHA, 12 containing EPA, 26 containing LA, and 68 containing ARA were identified in the lipidome assay of boiled egg yolks in the previous study, which represents a large difference. On the one hand, it is possible that the egg yolk lipids in the powdered state are more easily extracted by the solvent, thus enabling the detection of the corresponding lipid species. On the other hand, it is also possible that the spray-drying treatment resulted in the disruption of the structure of the protein-lipid complex in the egg yolk powder, which in turn caused more lipid freeing and complex changes in these PUFAs, but further studies are needed. The PUFAs found in egg yolks were mainly in the form of phospholipids and TGs, which is also consistent with previous findings. We also found that the forms of lipids containing DHA, EPA, LA and ARA in egg yolk changed significantly during spray drying processing and accelerated storage.

The total abundance of lipids containing DHA, EPA, LA, and ARA did not change significantly during the spray-drying process (SEY) compared to those in FEY ($p > 0.05$). However, the form of PUFAs present in the SEY compared with FEY samples changed considerably (Fig. 6A–D). Among them, the percentage of PE-DHA decreased significantly (from 64.99% to 60.04%), while the percentage of TG-DHA

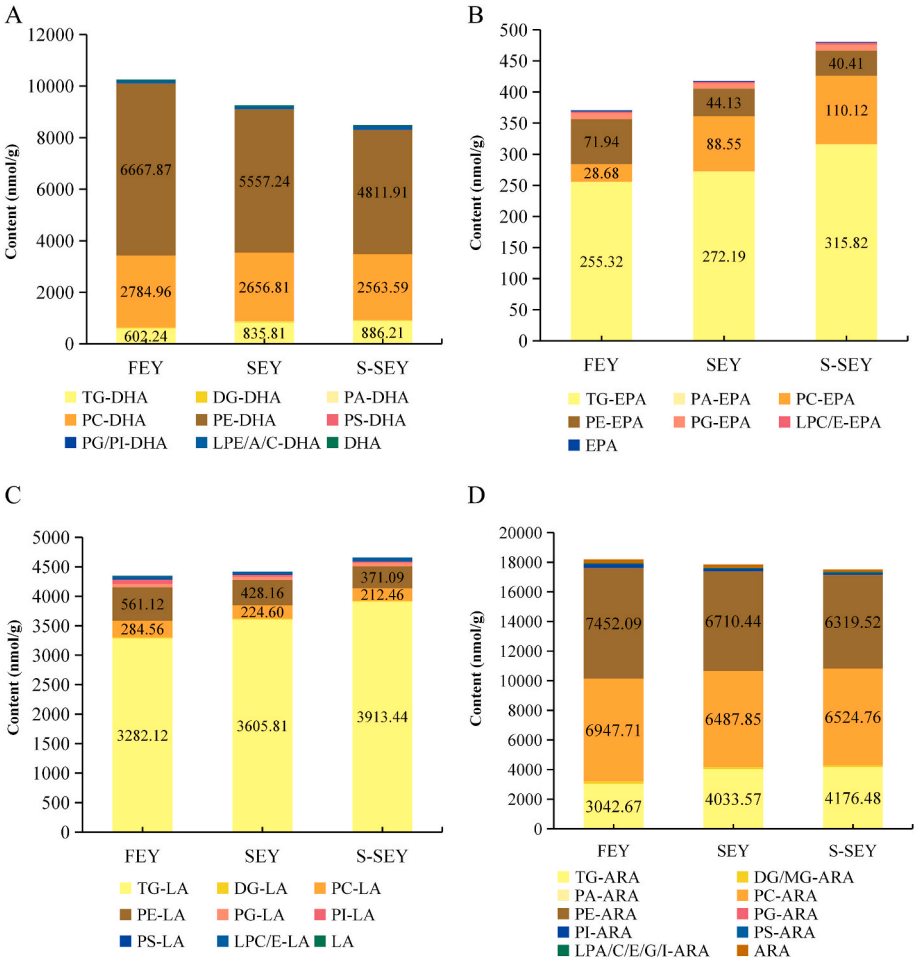

Fig. 6. Variation in the abundance values of different forms of polyunsaturated fatty acids (PUFAs) containing (A) DHA (C22:6), (B) EPA (C20:5), (C) LA (C18:3), and (D) ARA (C20:4). FEY, freeze dried egg yolk powder; SEY, spray-dried egg yolk powder; S-SEY, accelerated stored spray-dried egg yolk powder.

increased from 5.87% to 9.03% and the percentage of PC-DHA also increased from 27.15% to 28.70%, but resulting in a decrease in actual abundance (from 2784.96 to 2656.81 nmol/g). PE-EPA also showed a significant decrease (from 19.40% to 10.56%), PC-EPA increased from 7.73% to 21.19%, and TG-EPA increased in actual abundance (from 255.32 to 272.19 nmol/g), although it decreased in relative abundance (from 68.84% to 65.13%) in SEY samples. There was also a small decrease in PE-LA (from 12.91% to 9.70%) and PC-LA (from 6.55% to 5.09%), while the percentage of TG-LA slightly increased (from 75.54% to 81.66%) in SEY samples. There was also a decrease in PE-ARA (from 40.96% to 37.60%) and in PC-ARA (from 38.19% to 36.36%), whereas TG-ARA increased from 16.72% to 22.60% in the SEY compared with FEY samples.

The total abundance of lipids containing EPA, LA and ARA in the SEY also did not change significantly during accelerated storage ($p > 0.05$), but the abundance of lipids containing DHA in S-SEY was significantly reduced compared to that in FEY ($p < 0.05$). In addition to the changes in total abundance, the presence of PUFAs in the SEY also changed considerably in the form during accelerated storage (Fig. 6A–D). Among them, the percentage of PE-DHA in S-SEY decreased further (from 60.04% to 56.69%) compared to SEY, while the percentage of TG-DHA increased further from 9.03% to 10.44%, and the percentage of PC-DHA, although it continued to increase from 28.70% to 30.20%, further decrease in actual abundance occurred (from 2656.81 to 2563.59 nmol/g) in S-SEY samples. For EPA, the percentage of PE-EPA also decreased further significantly (from 10.56% to 8.40%), the percentage of TG-EPA increased from 65.13% to 65.73%, and the percentage of PC-EPA increased further from 21.19% to 22.92% in S-SEY samples. For LA, the percentage of PE-LA decreased further (from 9.70 to 7.97%) and PC-LA also decreased further from 5.09% to 4.56%, while the percentage of TG-LA increased further from 81.66% to 84.08% in S-SEY samples. For ARA, the percentage of PE-ARA in S-SEY decreased further (from 37.60% to 36.08%), the percentage of PC-ARA increased from 36.36% to 37.25%, while the percentage of TG-ARA increased further from 22.60% to 23.84% in the S-SEY samples compared with SEY samples.

Therefore, among the lipid species DHA, EPA, LA, and ARA, only the total abundance of DHA in egg yolk changed significantly during the spray-drying processing and/or accelerated storage. Moreover, the presence of some PUFA forms changed accordingly, where the abundance of PUFAs mainly found in the form of phospholipids continued to decrease, while the abundance of PUFAs in the form of TG continued to increase. This may affect the efficiency of digestion and absorption of spray-dried egg yolk powder products in the human gut. For example, it has been found that phospholipid forms of DHA and EPA are more beneficial for human metabolism than TG forms (Rossmeisl et al., 2012; Zhang et al., 2019). Finally, it will be important to further investigate whether the altered form of PUFAs present also has an effect on the processing characteristics of the yolk powder.

5. Conclusion

This study showed that spray-drying processing and subsequent accelerated storage processes have a serious effect on egg yolk lipids. Using freeze-dried egg yolk powder as a control, LC-MS/MS analysis and comparison revealed that spray-drying processing treatment caused a decrease in the total unsaturation of phospholipid species in egg yolk, as well as a decrease in FFAs such as arachidonic acid and linoleic acid and an increase in the corresponding oxidation products. In addition, spray-drying processing decreased the abundance of phospholipids such as PA, PC, and PE, with a corresponding increase in the lysophospholipids LPA, LPC, LPE. Thus, the spray-drying process caused the oxidation of egg yolk lipids and potential hydrolysis of phospholipids, and these reactions were aggravated during subsequent accelerated storage. This may be due to heat treatment induced changes in the structure of the protein-lipid complex in egg yolk, which in turn led to some fatty acid molecules becoming more susceptible to freeing, oxidation and hydrolysis. In addition, during spray-drying processing and accelerated storage, the presence of some PUFAs changed in form, which may affect the processing characteristics of egg yolk products and the absorption effect in the human intestine. However, further research and exploration is needed. Therefore, the results of this study provide theoretical support and data references for the industrial production of high-quality egg yolk products.

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CRediT authorship contribution statement

Wei Luo: Investigation, Data curation, Formal analysis, Writing – original draft. **Xialei Liu:** Investigation, Data curation, Formal analysis, Writing – original draft. **Beibei Wang:** Methodology, Resources. **Di Wu:** Methodology, Resources. **Jinqiu Wang:** Writing – review & editing, Project administration. **Fang Geng:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2023.100503.

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