



Lipidomics reveals the effect of hot-air drying on the quality characteristics and lipid oxidation of Tai Lake whitebait (*Neosalanx taihuensis* Chen)

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ABSTRACT

The present study aimed to investigate the impact of temperature on the quality attributes, particularly lipid oxidation and phospholipid molecular species, of Tai Lake whitebait during hot-air drying. Fresh samples of Tai Lake whitebait were subjected to heat treatment and were dried at temperatures of 40, 50, 60, and 70 °C for 12 h. The results indicated significant and continuous increases in peroxide values (POV), thiobarbituric acid reactive substances (TBARS), and saturated fatty acids (SFA) contents during hot-air drying, but reductions in mono-unsaturated fatty acids (MUFA). During the hot-air drying process, the mass fraction of triacylglycerol (TAG) and phospholipid (PoL) decreased, while the fractions of diacylglycerol (DAG), monoacylglycerol (MAG) and free fatty acid (FFA) increased. Furthermore, the drying treatment led to a more pronounced reduction in Phosphocholine (PC) content compared to phoethanolamine (PE). A total of 17 phosphatidylcholines (PC), 18 phosphatidylethanolamine (PE), 7 phosphatidylinositol (PI), and 9 phosphatidylserine (PS) molecular species were identified. By comprehensively considering the drying time and quality attributes, 50 °C was selected the ideal temperature for drying. The findings of the investigation revealed that thermal drying significantly influenced the quality attributes and lipid oxidation of Tai Lake whitebait. The insights gained from this study are valuable for improving the drying process for Tai Lake whitebait and enhancing its overall quality.

1. Introduction

The Tai Lake whitebait (*Neosalanx taihuensis* Chen), an important freshwater icefish in terms of economic importance, belongs to the family Salangidae of the order *Osmeriformes* (Wang et al., 2018). Its primary habitat is located in China, specifically in the offshore regions of eastern China, including the Yangtze River basin, where most of the 17 known species are found (Deng et al., 2023). Due to its remarkable commercial value, Tai Lake whitebait has been introduced into more than 20 provinces, making it the most widely distributed commercial icefish in China (Fang et al., 2022). In 2022, the production of Lake

whitebait in China reached 12,177 tons, according to the China Fishery Statistical Yearbook 2023. Lake whitebait is available in various forms such as soft-packed ready-to-eat leisure food, dried products, puffed food, and more (Xu et al., 2024).

Tai Lake whitebait is highly appreciated for its positive health effects, primarily attributed to its main lipid component and its abundance of long-chain polyunsaturated fatty acids Omega-3 (n-3 LC-PUFA). The abundance of phospholipids (PL) and n-3 LC-PUFA in Tai Lake whitebait makes it a valuable source of lipids with significant health benefits. Moreover, it is rich in essential nutrients such as thiamine, riboflavin, and nicotinic acid. Recognized by the global nutrition community as a

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nourishing food with potential longevity benefits, Tai Lake whitebait can be consumed whole. However, like other fresh fish products, whitebait is prone to spoilage and deterioration due to its high water content and the abundance of active endogenous enzymes (Lin et al., 2022). Therefore, immediate processing or freezing is recommended to maintain its quality and freshness. Whilst proper chilling and freezing can extend the shelf life of Tai Lake Whitebait, it can be costly and challenging to maintain quality over an extended period. Among various preservation techniques, drying plays a pivotal role in extending the shelf life of whitebait by inhibiting microbiological growth, enzyme activity, and chemical reactions, while minimizing quality loss. Drying also enhances the market value of dried products and reducing packaging, storage, and shipping costs. Consequently, several drying technologies, including microwave drying (MWD), cold-air drying (CAD), vacuum freeze drying (VFD), and hot-air drying (HAD), have been developed and applied to aquatic products (Pham, Martens, Karim, & Joardder, 2018; Piskov et al., 2020). Among these techniques, HAD is the most widely used method in the aquatic product processing industry due to its cost-effectiveness, short processing time, easy of control, thereby extending their shelf life and preserving their quality such as *Macra veneriformis* and *Ostrea talienwhanensis* (Wang et al., 2023; Zhao et al., 2021). However, compared to other preservation methods, hot-air drying can cause the degradation of several heat-sensitive compounds presene in seafood, including lipids and protein. The degradation of lipids during hot-air drying can be attributed to multiple factors, such as exposure to high temperatures, drying duration and oxidation reactions. However, to the best of our knowledge, the effects of temperature on the quality of Tai Lake Whitebait products by HAD are still unclear.

The aim of this study was to determine the effect of hot-air drying temperature (40, 50, 60, and 70 °C) on the quality and lipid oxidation of Tai Lake whitebait. Comprehensive analyses of the dried Tai Lake whitebait's acid value (AV), peroxide value (POV), thiobarbituric acid reactive substances (TBARS), fatty acid composition (FA), lipid class, triacylglycerol (TAG), phosphocholine (PC), phoethanolamine (PE), and variations in moisture contents were carried out. In addition, PCA was used to analyze the complicated results.

2. Materials and methods

2.1. Materials

Fresh Tai Lake whitebait (average length: 9.47 ± 0.35 cm, average weight: 8.87 ± 0.41 g) were purchased from Changxing Xintang Fishery Market (Changxing, China) in October 2022. The reference chemicals of phosphatidylinositol (PI 16:0/16:0), phosphatidylethanolamine (PE 15:0/15:0), phosphatidylcholine (PC 14:0/14:0), and phosphatidylserine (PS 14:0/14:0) were purchased from Avanti Polar Lipids, Co., Inc. (Alabaster, AL, USA). The standard solutions were prepared by diluting the reference chemicals to 1 mg/L with methanol/chloroform (1:1, v/v). The solvents of acetonitrile and methanol were purchased from Hangzhou Yunrui Reagent Co., Ltd. (Hangzhou, China). Ammonium formate and formic acid were bought from Hangzhou Jurui Reagent Co., Ltd. (Hangzhou, China). Ultrapure water (18.2 MΩ cm) was purified using a Milli-Q system (Millipore). Other chemicals and solvents were of analytical grade and obtained from Qihao Chemical Scientific Co., Ltd. (Hangzhou, China).

2.2. Hot-air drying process

A part of the raw materials was utilized as the fresh control (FC), and the rest was separated into four groups, which were heat-dried at 40, 50, 60 and 70 °C (HD-40, HD-50, HD-60 and HD-70, respectively) with PH-101(S) blast drying oven (Hengkuo Instrument Co., Ltd., Shanyu, Shaoxing, China) for 12 h with an air rate of 1.5 m/s to get dried samples. The dried samples were smashed using a RS-FS1401 pulverizer (Rongshida machinery Co., Ltd., Gangzhou, Guangdong, China). The

drying process was terminated when the moisture content was less than 12 g/100g, in accordance with the Chinese national standard for aquatic processing (NY/T 1712–2009).

The sample collection was done at regular intervals of 3 h for the purpose of determining POV, TBARS, and AV. The identification of lipid classes, phospholipid classes, FA, and phospholipid molecular species was determined at the beginning and the end of the drying at each temperature. Following collection, all samples were immediately kept at −30 °C.

2.3. Moisture content analysis

Moisture content of dried Tai Lake whitebait was determined according to the Neri's study with a few minor modifications (Neri, Jung, Jang, Kang, & Choi, 2021). Throughout the 12 h of the drying process at each temperature, the moisture content was measured every 3 h. The samples were dried at 105 °C in an air-drying oven (Hengkuo PH-101(S), Zhejiang, China), and the moisture content was calculated by the weight loss as a percentage of the beginning weight.

2.4. Lipid extraction

The method described in Bienkiewicz's study was used to extract total lipids from whitebait with minor modifications (Bienkiewicz, Tokarczyk, Czerniejewska-Surma, & Suryan, 2019). Briefly, a 50 g portion of whitebait powder was poured into a 500 mL Erlenmeyer flask, and then mixed with 50 mL chloroform and 100 mL methanol. After stirring for 1 h at 30 °C, 50 mL of chloroform and 50 mL of deionized water were added. After thorough mixing, the mixture was centrifuged using a high-speed refrigerator for 5 min at 10,000 g. To obtain the final lipids, the upper organic layer was collected, evaporated to a limited amount, and then dried at 30 °C with nitrogen. The lipids were collected and stored at −80 °C for later analysis.

2.5. Acid value (AV) analysis

The AV was carried out using the AOCS (2009) approach. Briefly, 12.5 mL of isopropanol was used to dissolve 0.10 g of fat. 25 mL of diethyl ether were added after mixing. Potassium hydroxide (0.01 mol/L) was then titrated into the solution. The amount of KOH consumed was used to calculate the AV, which was given as mg KOH/g lipid.

2.6. Peroxide value (POV)

The POV was quantified according to the AOCS approach with some modifications. Briefly, 0.10 g of lipid sample was thoroughly dissolved in 10 mL of isooctane, then 1 mL of saturated potassium iodide and 15 mL of glacial acetic acid was added. 30 mL of deionized water was added after 60 s of stirring at 30 °C. The material was thoroughly mixed before being titrated with sodium thiosulfate, 0.005 mol/L, using an automatic potentiometric titrator. The POV were calculated according to weight of lipid sample and volume of sodium thiosulphate. Results of the POV were represented as meq oxygen/kg.

2.7. Thiobarbituric acid reactive substances (TBARS) value

The TBARS was performed according to the procedure of Gang et al. with a slight modification (Gang et al., 2019). Briefly, 0.2 g lipid was mixed with 2 mL stock solution of 0.375 g/100 mL 2-thiobarbituric acid (TBA) and 15 g/100 mL trichloroacetic acid (TCA) in 0.25 mol/L hydrochloric acid solution (HCl), then heated at 90 °C for 20 min in a boiling water bath to develop a red color, then cooled in an ice bath and centrifuged at 6000 g for 15 min. Thermo Scientific Varioskan™ flash spectral scanning multimode reader (Thermo Scientific, Waltham, MA, USA) was then used to measure the absorbance of the upper phase at

532 nm. Stock solutions of MDA was used to generate the standard curve for the determination of TBARS, which is reported as mg MDA equivalents/kg lipid.

2.8. Total oxidation value (TOTOX)

The determination of TOTOX was conducted according to Xie using the following equation (Xie et al., 2018): $TOTOX = 2POV + TBARS$.

2.9. Fatty acid composition analysis (FA)

According to a prior work (Li et al., 2023), FA compositions were analyzed by using an Agilent 7890A Gas Chromatography-5977C MS system (Palo Alto, CA, USA) linked to an EI source and equipped with an Agilent HP-5-MS column.

In brief, 100 mg of lipid sample was mixed with 5 mL of 95 percent ethanol and 2.5 mL of KOH (500 mg/mL). After 2 h in a water bath at 60 °C, the mixture was immediately cooled to 20 °C. The lipid phase was extracted six times with hexane, each time using 3 mL of hexane to remove the unsaponifiables. The system was then acidified with 6 mol/L HCl, and FFA was extracted six times using 3 mL of hexane at a time. The hexane extracts were mixed and dried with nitrogen flow at 35 °C. The residue was dissolved in hexane to 10 mg/mL and methylated in a vial with HPLC-grade methanol containing 0.1 percent H₂SO₄. After 1 h of interaction in a 70 °C water bath, the mixture was cooled to room temperature and 1 mL of distilled water was added. The FA methyl ester-bearing upper layer was collected and transferred to a new vial containing anhydrous sodium sulfate. The upper layer was recovered after an 12 h of storage at 4 °C, and analyzed by GC-MS. The temperature of the column was as follows: 50 °C for 1 min, raised to 200 °C at a rate of 50 °C/min, raised to 280 °C at a rate of 5 °C/min, increased to 310 °C at a rate of 15 °C/min, and maintained at 310 °C for 5 min. The injection volume was 1 µL. The ion's scan range was set to 50–550 m/z. By comparing mass spectra and relative retention durations with the NIST08 mass spectral database and authentic standards, the fatty acid methyl esters were identified.

2.10. Lipid class composition analysis

The Iatroscan MK-6S thin-layer chromatography analyzer (TLC-FID) was used to determine the lipid class composition in the lipids of Tai Lake whitebait lipid (Copeman et al., 2022). The ratio of the area of each peak to the sum of all peak areas was used to calculate the mass percentage of the total lipid class composition.

2.11. Phosphocholine (PC) and phoethanolamine (PE) contents

According to the Li procedure (Li et al., 2020), the quantitative analyses of PC and PE were detected using an ELSD 6000 detector and an LC-20AD HPLC system. The PE and PC contents of the dry Tai Lake whitebait were determined using the GPETn (C16:0/18:1) and GPCho (C16:0/18:1) standard curves. mg/g dry Tai Lake whitebait was used to express the results.

2.12. Phospholipids determination

The differentially processed whitebait samples were homogenized individually, and the phospholipids inside were extracted using a classic Folch method with modification (Xu et al., 2023). Briefly, the homogenized whitebait sample was weighed 0.1 g precisely, and 2 mL of a binary solvent mixture of chloroform and methanol (2:1, v/v) were added. The mixture was vigorously blended under ultrasound at 20 kHz and 100W for 10 min. The mixture was then shaken with the addition of 2 mL of water, then centrifuged at 8000 g for 15 min. The residue was twice more extracted with chloroform (1 mL) before the lower organic phase was recovered. Prior to HILIC-MS injection, the organic phases

were mixed, dried under nitrogen flow, redissolved in acetonitrile (5 mL), and filtered through a 0.22 µm membrane. To reduce the risk of lipid oxidation and hydrolysis, it was advised to carry out the entire phospholipid extraction process at ≤ 4 °C.

The phospholipids study was completed using a tandem mass spectrometer (4000Q-Trap, Applied Biosystems, CA). Polypropylene glycol, reserpine, and a ProteoMass TM peptide kit (AB Sciex, Foster City, CA) were used in accordance with the manufacturer's instructions to condition and calibrate the device prior to injection. The phospholipids were separated on a HILIC column (150 × 4.6 mm, 3 µm particle size) with temperature adjusted at 30 °C using a portion of 2 µL sample for each running. Following that the column was then flushed at a rate of 0.5 mL/min with mobile phase A (10 mmol/L ammonium formate and 10 mmol/L formic acid in water) and B (20 mmol/L formic acid in ACN). The profile is as follows: 0–3 min, A: B = 5: 95; 3–18 min, A: B = 5: 95 to A: B = 30: 70; 18–23 min, A: B = 30: 70 to A: B = 50: 50; 23–28 min, A: B = 50: 50; 28–29 min, A: B = 50: 50 to A: B = 5: 95; 29–32 min, A: B = 5: 95. The column was washed with A: B = 5: 95 once all of the target analytes had been eluted, and the HILIC condition was then changed to the starting condition for equilibration. The solvent was evaporated at 500 °C in electrospray ionization (ESI) ion source in negative-ion mode with the HILIC eluates flowing directly to the MS. The HILIC eluates were directly flowed to mass spectrometer (MS), and the solvent was evaporated at 500 °C in the negative-ion mode of an electrospray ionization (ESI) ion source. The decluttering potential (DP) and collision energy (CE) were set at –60 and –30 eV, respectively, while the ion source voltage was set at –4500 V. Curtain gas 35 psi, GS1 50 psi, and GS2 60 psi were the nitrogen gas's specifications. Analyst 1.6.3 (Applied Biosystems, CA) was used for instrument control and data gathering.

2.13. Statistical analysis

All the experimental data were represented by mean ± standard deviation. Statistical significance through the Duncan's post-hoc test was measured by IBM Inc.'s SPSS 26.0 software (Chicago, USA), the values of $p < 0.05$ were considered significant. The graphs in the document were generated using OriginLab Inc.'s OriginPro 2018 software (Northampton, USA).

3. Results and discussion

3.1. Changes in moisture content

The changes in moisture contents of Tai Lake whitebait during heat drying under different temperatures were shown in Table 1s. Tai Lake whitebait had an initial moisture content of 84.60 ± 0.95 g/100g, which decreased in a time-dependent manner after hot-air drying. For HD-40, the moisture contents after 3, 6, 9, and 12 h were 45.02 ± 0.42 g/100g, 19.97 ± 0.21 g/100g, 13.05 ± 0.21 g/100g, and 10.88 ± 0.15 g/100g ($p < 0.05$), respectively. As for HD-70, the corresponding values were 24.76 ± 0.29 g/100g, 10.81 ± 0.21 g/100g, 9.79 ± 0.19 g/100g, and 9.75 ± 0.14 g/100g, respectively. Because of the higher evaporation rate, the drying rate of Tai Lake whitebait with hot air was faster than with cold air. After drying for 6 h at HD-50, the moisture content decreased significantly to 11.91 ± 0.23 g/100g, compared to HD-40. After drying for 12 h at HD-70, the moisture content of dried Tai Lake whitebait reduced from 84.6 g/100g to 9.75 g/100g. The moisture content was 10.81 g/100g after only 6 h of drying. After 6, 9, and 12 h of drying, the moisture content of Tai Lake whitebait did not vary considerably. It was consistent with other studies, the drying rate was initially fast, and most of the moisture was lost within the first 6 h, resulting in a slower drying rate thereafter (Choopan, Panpipat, Nisoa, Cheong, & Chaijan, 2021).

Table 1

Changes in the relative content of fatty acid of Tai Lake whitebait during different drying temperatures.

Fatty acids	Raw	HD-40	HD-50	HD-60	HD-70
C14:0 (Myristic)	2.98 ± 0.27a	3.62 ± 0.30a	3.18 ± 0.06 ab	3.01 ± 0.18a	2.79 ± 0.17a
C15:0 (Pentadecanoic)	1.15 ± 0.13c	1.27 ± 0.08bc	1.57 ± 0.18 ab	1.51 ± 0.06bc	1.93 ± 0.24a
C16:0 (Palmitic)	20.89 ± 0.09e	21.49 ± 0.14d	22.13 ± 0.10c	22.45 ± 0.10b	22.85 ± 0.13a
C16:1 (Palmitoleic)	6.88 ± 0.05a	7.00 ± 0.21a	6.32 ± 0.41a	6.32 ± 0.58a	6.36 ± 0.24a
C17:0 (Margaric)	1.77 ± 0.06 ab	1.30 ± 0.04c	1.69 ± 0.04b	1.87 ± 0.07a	1.79 ± 0.08 ab
C18:0 (Stearic)	8.70 ± 0.28b	9.03 ± 0.37 ab	9.47 ± 0.07a	9.51 ± 0.08a	9.59 ± 0.26a
C18:1 (Elaidic)	12.72 ± 0.27 ab	13.26 ± 0.07a	12.63 ± 0.41 ab	12.43 ± 0.39b	12.15 ± 0.21b
C18:2 (LA)	1.73 ± 0.52	2.07 ± 0.38	1.13 ± 0.15	nd	nd
C18:3 (ALA)	3.54 ± 0.37a	3.36 ± 0.33a	3.41 ± 0.09a	3.20 ± 0.13a	3.27 ± 0.21a
C18:4 (Octadectetraenoi)	2.76 ± 0.14a	2.70 ± 0.14a	1.39 ± 0.29b	1.33 ± 0.38b	1.57 ± 0.06b
C20:1 (Eicoseni)	2.27 ± 0.13d	2.07 ± 0.07d	2.72 ± 0.08c	3.09 ± 0.07b	3.33 ± 0.09a
C20:2 (Eicosadienoic)	4.30 ± 0.12a	3.70 ± 0.49a	3.63 ± 0.09a	4.33 ± 0.30a	4.16 ± 0.46a
C20:3 (g-Eicosatrienoic)	5.94 ± 0.51b	6.49 ± 0.44 ab	6.88 ± 0.18 ab	7.27 ± 0.44a	7.13 ± 0.55a
C20:4 (AA)	2.32 ± 0.06b	1.53 ± 0.26c	2.84 ± 0.11 ab	3.05 ± 0.15a	2.79 ± 0.36 ab
C20:5 (EPA)	13.05 ± 0.27a	12.47 ± 0.10b	12.32 ± 0.06bc	12.18 ± 0.13bc	12.00 ± 0.09c
C22:6 (DHA)	5.38 ± 0.09a	4.67 ± 0.18b	4.60 ± 0.09b	4.46 ± 0.10b	4.41 ± 0.15b
C22:2 (Docosadienoi)	3.62 ± 0.14a	3.96 ± 0.28a	4.10 ± 0.25a	3.99 ± 0.28a	3.88 ± 0.23a
PUFA	21.87 ± 0.37a	22.33 ± 0.20a	21.67 ± 0.69a	21.85 ± 0.97a	21.84 ± 0.22a
MUFA	42.63 ± 0.26a	40.96 ± 0.43b	40.29 ± 0.69bc	39.81 ± 1.12bc	39.22 ± 0.54c
SFA	35.49 ± 0.20d	36.71 ± 0.49c	38.04 ± 0.14b	38.34 ± 0.18 ab	38.95 ± 0.35a

Data are mean ± SD values. Different letters represent significant differences ($p < 0.05$) on the same row.

3.2. Changes in POV, TBARS, TOTOX and AV during hot-air drying

POV, TBARS, and TOTOX values are indicators of the oxidation of lipids. POV, as an indicator of the formation of primary oxidation products, can be used to indicate the amount of peroxides in lipids, and is widely used to measure the degree of oxidation and rancidity of lipids (Qu, Wang, Wang, Yu, & Wang, 2020). As shown in Fig. 1a, the POV of fresh Tai Lake whitebait was 20.63 ± 0.50 meq oxygen/kg lipid, and when dried at 40, 50, 60 and 70 °C for 12 h, the POV of dried whitebait were 59.59 ± 0.59 , 67.25 ± 1.85 , 85.24 ± 1.79 , and 87.44 ± 1.50 meq oxygen/kg lipid (an increase of approximately 1.89-fold, 2.26-fold, 3.13-fold, and 3.24-fold, $p < 0.05$), respectively. The above results showed that higher drying temperature could produce more peroxide and increased the oxidation rate of Tai Lake whitebait lipid, moreover, the extending drying time also contribute to larger POV values. Similar results have been reported upon drying treatment of scallop (*Argopecten irradians*) adductor muscle (Xie et al., 2018). The results suggest that a

slight lipid oxidation developed in the shrimp muscle after the drying process, which may be due to the free radical formation increase during the heat treatment (Xie et al., 2018).

The TBARS value is a typical measurement for the degree of lipid secondary oxidation, particularly in aquatic products with a high fat content (Zhu et al., 2021). As shown in Fig. 1b, the TBARS value of fresh Tai Lake whitebait was 3.69 ± 0.84 mg MDA/kg sample, which increased significantly with the increase of drying temperature and time ($p < 0.05$), indicating that lipid oxidation products accumulated gradually during heat drying. The TBARS value of the HD-60 and HD-70 was significantly higher at the initial stage than that of HD-40 and HD-50 ($p < 0.05$). Therefore, in the early drying stage, the TBARS value of whitebait is higher at higher temperature. The content of TBARS in Tai Lake whitebait oil began to decline after drying at 60 and 70 °C for 9 h. However, the degree of lipid oxidation reaction in the late drying stage at 40 and 50 °C still demonstrated a sharp increase. Similar results were obtained by Shen et al. (Shen et al., 2023), the lipid content of fish oil is

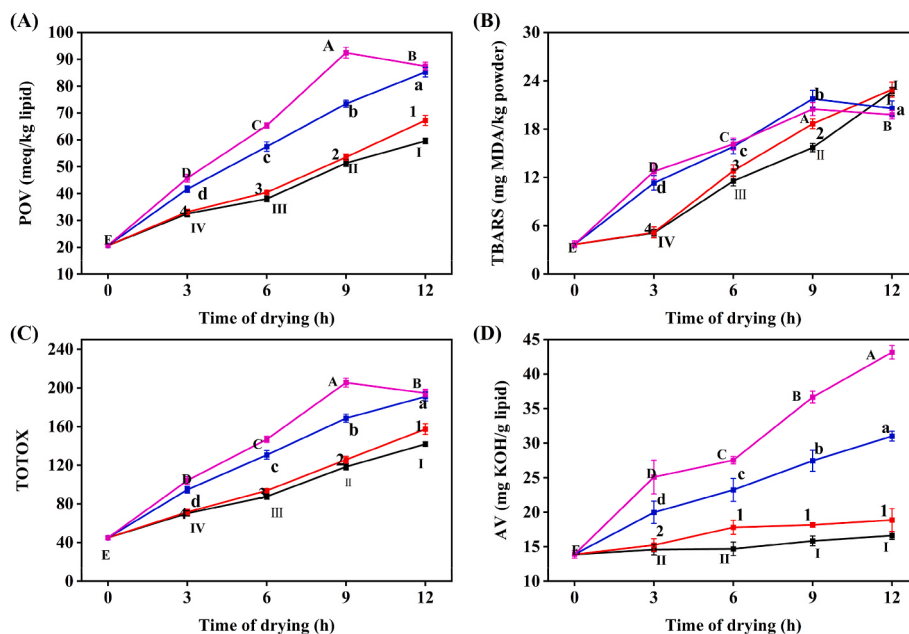


Fig. 1. (A–D) Changes of lipid stability indicators including AV, POV, TBARS, and TOTOX of Tai Lake whitebait samples during processing. ■: Tai Lake whitebait samples dried at 40 °C, ■: Tai Lake whitebait samples dried at 50 °C, ■: Tai Lake whitebait samples dried at 60 °C, ■: Tai Lake whitebait samples dried at 70 °C.

abundant in long-chain polyunsaturated fatty acids such as DHA and EPA, which are prone to oxidation to increase the TBARS value and generate malondialdehyde (MDA). However, MDA is unstable at high temperatures, which further oxidized to form volatile compounds or reacted with amino-containing compounds at a faster rate after formation.

The degree of oxidation of lipids can be determined using the TOTOX value, which serves as an indicator of both primary and secondary oxidation products. Hot-air drying considerably affects lipid oxidation, as seen in Fig. 1c. At 40 °C, 50 °C, 60 °C, and 70 °C, the TOTOX value was increased noticeably to 141.84 ± 2.43 , 157.41 ± 5.46 , 191.07 ± 4.56 and 194.66 ± 3.78 ($p < 0.05$), respectively.

The AV indicates the level of free fatty acids in lipids, which is the main indicator of lipids rancidity (Qu, Li, Yang, Wang, & Wang, 2022). The changes of lipid AV of whitebait are shown in Fig. 1d. The level of AV in the oil extracted from the untreated Tai Lake whitebait was 13.88 ± 0.55 mg KOH/g lipid. For hot-air drying at 40 °C, the AV at 3, 6, 9, and 12 h were 14.59 ± 0.76 , 14.68 ± 0.97 , 15.84 ± 0.71 , and 16.61 ± 0.55 mg KOH/g lipid, respectively. Drying the Tai Lake whitebait did not

cause statistically significant changes in the AV at 40 °C, but after drying at 70 °C for 12 h the AV increased to 43.15 ± 0.98 mg KOH/g (2.1-fold, $p < 0.05$). The results indicated that the higher temperature hot-air drying had a negative effect on the AV of Tai Lake whitebait lipids compared with the lower temperature drying. The lipid hydrolysis reaction caused the decomposition of glycerol ester to produce rancidity, which lead to the formation of free fatty acids in Tai Lake whitebait lipids. Temperature has a direct impact on the production of free fatty acids. Free fatty acids are more likely to develop at higher temperatures. Similar to the present study, TAG, PC and PE but increased AV in shrimps, indicating the occurrence of lipid hydrolysis (Li et al., 2020).

3.3. Fatty acid analysis

The changes of fatty acids (FAs) content during accelerated storage of Tai Lake whitebait were shown in Table 1. 17 species of fatty acids, including 5 types of SFAs (saturated fatty acids), 3 types of MUFAs (monounsaturated fatty acids), and 9 types of PUFAs (polyunsaturated fatty acids) were detected. Tai Lake whitebait contained a high

Table 2
The identity and content of each PC, PE, PI and PS molecular species ($\mu\text{g}\cdot\text{g}^{-1}$).

	m/z	Fatty acyl group	Molecular formula	Raw fish	HD-40	HD-50	HD-60	HD-70
PC [M + HCOOH]-	762.8	O-32:1	C40H80NO7P	59.93	56.85	32.16	22.72	26.12
	776.8	32:1	C40H78NO8P	75.96	76.07	67.53	68.99	53.85
	778.8	32:0	C40H80NO8P	46.19	35.5	50.13	36.82	21.95
	790.8	O-34:1	C42H84NO7P	65.32	52.47	54.94	36.17	45.65
	802.8	34:2	C42H80NO8P	47.72	58.97	56.8	34.81	28.72
	804.8	34:1	C42H82NO8P	226.25	225.29	218.24	216.05	141.29
	806.8	34:0	C42H84NO8P	48.04	44.2	50.81	34.64	20.74
	824.8	36:5	C44H78NO8P	124.09	129.16	96.18	105.44	77.78
	826.8	36:4	C44H80NO8P	144.92	144.38	154.71	122.48	91.89
	828.8	36:3	C44H82NO8P	57.84	42.86	44.78	35.46	25.00
	836.8	O-38:6	C46H82NO7P	50.17	41.85	48.15	28.17	18.6
	850.8	38:6	C46H80NO8P	187.7	178.02	168.35	148.01	94.1
	852.8	38:5	C46H82NO8P	138.11	128.53	145.17	112.07	67.01
	854.8	38:4	C46H84NO8P	51.09	48.87	52.45	38.89	25.42
	876.8	40:7	C48H82NO8P	48.38	39.11	43.65	38.37	20.46
	878.8	40:6	C48H84NO8P	68.5	56.9	62.37	47.6	25.7
	898.8	42:10	C50H80NO8P	43.89	47.28	45.53	36.7	18.04
PE [M-H]-	716.8	34:1	C39H76NO8P	54.16	50.89	43.74	26.26	25.07
	746.8	36:0	C41H82NO8P	84.07	79.29	51.48	28.98	46.28
	748.8	O-38:6	C43H76NO7P	48.04	55.65	31.82	31.35	21.46
	750.8	O-38:5	C43H78NO7P	30.06	32.42	49.89	54.46	9.7
	762.8	38:6	C43H74NO8P	110.51	109.28	61.81	43.74	50.25
	764.8	38:5	C43H76NO8P	53.51	54.66	36.59	21.49	33.21
	774.8	38:0	C43H86NO8P	63.58	46.52	26.66	15.1	23.62
	776.8	O-40:6	C45H80NO7P	140.07	146.42	129.93	132.71	103.6
	778.8	O-40:5	C45H82NO7P	85.18	68.36	96.45	70.84	42.24
	788.8	O-40:0	C45H76NO8P	35.11	29.63	37.98	29.83	20.9
	790.8	40:6	C45H78NO8P	101.26	100.94	105.7	69.95	47.89
	792.8	40:5	C45H80NO8P	37.98	45.92	46.26	17.5	27.05
	802.8	40:0	C45H90NO8P	87.99	113.45	109.22	67.07	55.29
	830.8	42:0	C47H94NO8P	61.80	49.70	52.48	46.89	25.62
	834.8	44:12	C49H74NO8P	56.47	52.08	48.72	35.31	29.6
	836.8	44:11	C49H76NO8P	38.15	40.96	92.59	54.26	18.41
	838.8	44:10	C49H78NO8P	36.05	54.86	70.15	47.31	37.18
	840.8	44:9	C49H80NO8P	39.51	36.59	39.17	28.59	20.9
PI [M-H]-	835.9	34:1	C43H81O13P	100.70	132.83	46.11	14.47	10.35
	837.9	34:0	C43H83O13P	47.45	60.04	11.37	1.71	19.14
	881.9	38:6	C47H79O13P	53.69	91.29	47.41	30.62	21.11
	883.9	38:5	C47H81O13P	57.50	59.39	54.31	28.79	18.25
	885.9	38:4	C47H83O13P	111.12	105.75	97.67	94.68	51.10
	909.9	40:6	C49H83O13P	241.06	287.78	214.86	121.76	71.48
	911.9	40:5	C49H85O13P	65.68	108.22	54.12	30.57	27.23
PS [M-H]-	760.8	34:1	C40H76NO10P	20.90	15.76	14.52	2.03	6.61
	788.8	36:1	C42H80NO10P	44.55	22.71	4.20	2.71	2.03
	810.8	38:4	C44H78NO10P	17.45	9.62	3.08	2.07	5.08
	834.8	40:6	C46H78NO10P	93.95	94.44	33.07	7.17	5.96
	836.8	40:5	C46H80NO10P	43.76	46.02	35.63	32.91	23.07
	838.8	40:4	C46H82NO10P	20.34	35.9	1.99	2.19	9.58
	880.8	44:11	C50H76NO10P	24.64	41.75	6.93	8.06	8.62
	884.8	44:9	C50H80NO10P	48.29	51.87	40.78	41.75	17.01
	886.8	44:8	C50H82NO10P	95.28	90.89	68.21	70.25	29.26

proportion of MUFAs (39.22–42.63 percent of total FAs), followed by SFAs (35.49–38.95 percent of total FAs) and PUFAs (21.67–22.33 percent of total FAs).

In comparison to the content of PUFAs, the relative amount of MUFA was much larger ($p < 0.05$). Eicosapentaenoic acid (EPA, C20:5, 12.00–13.05 percent of total FAs) was the most prevalent PUFA, and palmitic acid (C16:0, 20.89–22.85 percent of total FAs) was the principal SFA, while elaidic acid (C18:1, 12.15–13.26 percent of total FAs) was the dominant MUFA. The fatty acid composition changed clearly under different temperatures, with a significant increase of SFA and a decrease of PUFA, especially the contents of EPA and DHA ($p < 0.05$). Among SFA, the content of palmitic acid was increased by 0.03-fold under the temperature 50 °C and 0.09-fold under 90 °C. The MUFA content decreased after the drying at the range. Indeed, the degree of unsaturation is closely associated to the rate of FA breakdown, and PUFA, particularly EPA and DHA, showed greater sensitivity than other fatty acids (Biancolino, Parlapiano, Denti, Di Nardo, & Prato, 2021). Together, these findings showed that the drying process caused oxidative degradation in PUFA in Tai Lake whitebait.

3.4. Changes of lipid class compositions

The changes of lipid composition of Tai Lake whitebait during hot-air drying are shown in Table 2s. Among all lipids, TAG was the most abundant lipid in fresh whitebait. PoL was the second main component. It also contains a small amount of FFA, CHO, DAG and MAG. During the hot-air drying process, the mass fraction of TAG and PoL decreased, while the mass fraction of DAG, MAG and FFA increased. For example, in the samples of hot-air drying at 40, 50, 60 and 70 °C, the percentages of TAG decreased 0.05-fold, 0.08-fold, 0.20-fold, and 0.23-fold, respectively, and the percentages of PoL decreased 0.37-fold, 0.41-fold, 0.52-fold, and 0.57-fold, respectively. The results of this study have a similar trend to that of a previous study on dried clams (Xie et al., 2018). Meanwhile, in the samples of HD-70, the percentages of FFA, CHO, DAG, and MAG increased 0.19-fold, 0.06-fold, 0.02-fold, and 0.01-fold, respectively.

Obviously, the higher drying temperature caused a larger decrease in TAG and PoL contents. The results showed that TAG and PoL were hydrolyzed during processing, which was consistent with the lipid changes of shrimp (*Acetes vulgaris*) during hot-air drying. These changes may be explained by lipid hydrolysis and FFA release caused by endogenous and microbial lipases and phospholipases. Unsaturated fatty acids were the most sensitive components in lipid oxidation. With the increase of FFA during the processing, the progression of lipid oxidation will be accelerated.

3.5. Phosphocholine (PC) and phosphoethanolamine (PE) contents

The PC and PE contents of fresh Tai Lake whitebait were 7.91 ± 0.13 and 6.52 ± 0.10 mg/g (on a dry weight basis), respectively, with a temperature-dependent decrease under different drying temperature (Fig. 1s). For PC, the content decreased to 6.29 ± 0.09 at 40 °C (a decrease of 0.20-fold), and further declined to 5.69 ± 0.10 , 4.73 ± 0.08 , 3.89 ± 0.13 under 50, 60, and 70 °C (decrease of 0.28-fold, 0.44-fold and 0.56-fold), respectively. As for PE, the content dropped to 5.79 ± 0.16 at 40 °C (a decrease of 0.11-fold), and declined to 5.01 ± 0.04 , 4.12 ± 0.08 , 3.14 ± 0.16 under 50, 60, and 70 °C (decrease of 0.23-fold, 0.37-fold and 0.52-fold), respectively. The higher drying temperature caused larger reduction in the contents of PC and PE in all the samples ($p < 0.05$). Furthermore, the drying treatment resulted in a greater decrease in the content of PC compared to PE, indicating that the phospholipids in Tai Lake whitebait might further be hydrolyzed during the drying process. Previous studies also reported that heat drying played an important role in lipid hydrolysis of fish (Córdova Murueta, Navarrete del Toro, & García Carreño, 2007).

3.6. Phospholipid identification

The effect of drying temperature on the lipid peroxidation of Tai Lake whitebait was investigated by monitoring the content of phospholipids. The variation of each phospholipid molecular species under different processing temperature (40, 50, 60, and 70 °C) was depicted in Fig. 2.

The phospholipid composition of the processed whitebait was analyzed using HILIC-MS that achieve phospholipids separation in a single running. By referring to the retention behavior of phospholipid standards, the phospholipid classes in whitebait extract can be unambiguously identified. As shown in Fig. 2A, the PC and PE were eluted at 11.11 min and 12.00 min, respectively, while the PI & PS were co-eluted resulting in an overlapped peak at 16.40 min. It can be directly observed that PC showed the largest peak area followed by PE, while PI & PS only produced a minor peak. To ascertain the phospholipid molecular species, the chromatographic peaks were integrated to obtain the mass spectrum of each phospholipid class. In Fig. 2B, the PC showed a large variety in the m/z range of 750–950, which were adducted as $[M + HCOO]^-$ due to the existence of ammonium formate as an additive in mobile phase. The ion peaks corresponded to different PC molecular species, depending on the variation of length and number of double bonds in fatty acyl chains at the glycerol backbone. The identity of each ion peak was characterized using database of Lipid MS Predictor program. Taking the ion of m/z 804.8 as an example, the value was input into the software which could provide the probable attributions, including PC 34:1 (C42H81NO8P⁻) and O-PC 35:1 (C43H85NO7P⁻). The latter was excluded because only the attribution with even numbered radical carbons was considered. Furthermore, the sn1/sn2 composition was speculated among several possibilities, such as PC (12:0/22:1), PC (14:0/20:1), PC (16:0/18:1), PC (16:1/18:0), and etc. Finally, the ion of m/z 804.8 was identified as PC (16:0/18:1) by referring to the results of fatty acid study, MS2 analysis, and previous publications (Shen, Li, et al., 2020; Song et al., 2019; Yu et al., 2020). The rest PC molecules were also identified, and there was a total of 17 PC molecular species characterized. For quantitation, the peak area of each

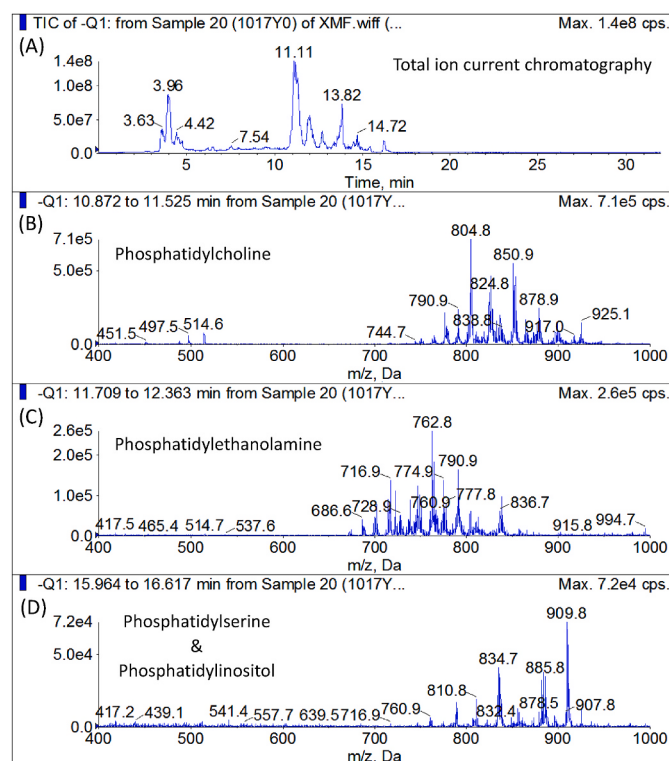


Fig. 2. HILIC-MS of the phospholipid composition of the processed whitebait.

PC molecular species was acquired using the function of extracted-ion chromatography (XIC), and its content was calculated through the calibration models established in our previous publications (Shen, Song, et al., 2020; Yu et al., 2020). The identity and content of each PC molecular species were summarized in Table 2, from where it could be directly observed that the m/z 804.8 (PC (16:0/18:1), 226.25 µg/g) was the most abundant ion, followed by the m/z 850.8 (PC (18:1/20:5), 187.7 µg/g), the m/z 826.8 (PC (16:0/20:4), 144.9 µg/g), the m/z 852.8 (PC (18:1/20:4), 187.7 µg/g), and etc. There were many suspected EPA/DHA structured phospholipids detected, such as the 824.8 (PC (16:0/20:5), 124.09 µg/g), the m/z 876.8 (PC (20:2/20:5), 68.5 µg/g), the m/z 898.8 (PC (20:4/22:6), 43.89 µg/g), etc. It was reported that EPA/DHA structured phospholipids could alleviate obesity-related metabolic disorders, lower plasma norepinephrine concentrations (Du et al., 2015; Kei Hamazaki et al., 2005; Liu et al., 2014). There were also plasmalogen (vinyl ether) and plasmalogen (alkyl ether) phospholipids (O-PC) observed like the m/z 790.8 (O-PC (16:0/18:1), 65.32 µg/g) and the m/z 836.8 (O-PC (16:1/20:5), 50.17 µg/g). According to a report, plasmalogens have a role in vesicle formation, ion transport, and the production of secondary signal mediators, as well as being linked to a variety of risk factors for metabolic syndrome, atherosclerosis, etc (Wu et al., 2019). In the same way, there was a total of 18 PE molecular species identified. The m/z 776.8 (O-PE (20:1/20:5), 140.07 µg/g) and the m/z 790.8 (PE (20:1/20:5), 101.26 µg/g) were the major ions observed in the spectrum of PE (Fig. 2C). It was reported that EPA structured phospholipids could improve Aβ-induced cognitive deficiency in Alzheimer's disease (AD) rat models. For PI and PS, there were 7 and 9 molecular species, respectively. The representative PI and PS molecular species were the m/z 909.9 (PI (20:1/20:5), 241.06 µg/g) and the m/z 834.8 (PS (20:1/20:5), 93.95 µg/g), both of which were EPA containing phospholipids (Fig. 2D).

Under oxidative stress, free radicals attack cellular membranes, lipoproteins, and other molecules that contain unsaturated lipids. This causes oxidative damage known as lipid peroxidation, which leads to the creation of many metabolites and polymerization products. Long-term studies on lipid peroxidation have recently attracted increased interest from the dietary and medicinal perspectives. The effect of drying temperature on the lipid peroxidation of Tai Lake whitebait was investigated by monitoring the content of phospholipids. The variation of each phospholipid molecular species under different processing temperature (40, 50, 60, and 70 °C) was depicted in Table 2. The content of PC molecules was kept constant and/or decreased slightly ($p > 0.05$) when the temperature went up to 50 °C. When the dry temperature was further increased to 70 °C, the contents of some PC molecules were dropped significantly ($p < 0.05$), such as the m/z 850.8 (PC (18:1/20:5)), m/z 826.8 (PC (16:0/20:4)), m/z 836.8 (O-PC (16:1/20:5)), etc. Such deterioration and oxidation effect became more serious at 70 °C. The PE molecules behaved similar variation trends to the PC as shown in Table 2. The contents of PE molecules were stable ($p > 0.05$) in raw fish and 40 and 50 °C treated Tai Lake whitebait samples. The deterioration took place when the temperature was 60 °C, as indicated by the great ($p > 0.05$) reduction of the contents of PE molecules, and this phenomenon became more significant along with the further increased temperature. The variation trends in Table 2 indicated that the molecular species of PI and PS were more vulnerable to free radicals than PC and PE, because some molecules were sensitive to temperature and their degradation initiated at 50 °C, for example, the m/z 835.9 (PI (16:0/18:1)), m/z 837.9 (PI (16:0/18:0)), m/z 834.8 (PS (20:1/20:5)), m/z 886.8 (PS (22:5/22:6)), etc. There were also some molecular species kept constant and stable along with the increased temperature, such as the m/z 885.9 (PI (18:1/20:3)), the m/z 836.8 (PS (20:0/20:5)), and the m/z 884.8 (PS (22:3/22:6 & 22:4/22:5)). As a conclusion, the temperature of 50 °C was selected the ideal temperature for drying, under which the contents of phospholipid molecules were constant and/or only slightly reduced ($p < 0.05$).

Phospholipids are the main source of polyunsaturated fatty acids in

Tai Lake whitebait, and their content may be associated with the drying temperature. Tai Lake whitebait has been found to contain higher levels of total phospholipids, with substantial amounts of essential fatty acids and arachidonic acid in the phospholipid fraction, which contribute to its nutritional and medicinal value. The consumption of phospholipids can inhibit blood clotting, activate the body's defense mechanisms, and enhance phagocytosis, thereby regulating physiological functions. Glycerophospholipids, as the major lipid component in all biological membranes, play a crucial role in the structure, function, formation, and signaling of cellular membranes in all animal cells. Shen et al. reported the total contents of the four detected phospholipid classes (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol) in three fish species, *Lateolabrax japonicus*, *Ctenopharyngodon idellus*, and *Carassius auratus*, ranging from 1.52 to 3.29 mg/g (Shen et al., 2012). They also reported the content of 20 species of PC, 22 species of PE, 15 species of PS, and 5 species of PI in *Hypophthalmichthys nobilis*, which were 224.1, 124.1, 27.4, and 34.7 µg/g, respectively (Jin et al., 2017). Clearly, lipids play important roles in human physiology, with varying effects. Tai Lake whitebait contains a considerable amount of lipids, and its content varies with different drying temperatures. Therefore, greater attention should be paid to the potential adverse effects of drying conditions on the processing of Tai Lake whitebait.

3.7. PCA

A visual comparison between samples treated by different temperature can be made by plotting the main two PC scores calculated from variables defined by moisture, TBARS, POV, PC, PE and phospholipid molecular species data. The score plot was shown in Fig. 3 using PC1 (70.0%) and PC2 (15.9%), with the identification result reaching 85.9%, indicating that there is a strong relation between the heat treatment conditions and the lipid quality of the Tai Lake whitebait. It also showed that significant differences of the different temperature on the quality of Tai Lake whitebait. It further revealed that the distance between the HD-40 group and the raw group, the HD-50 group and the raw group were the closest, indicating that their lipid composition was similar, as the temperature increased, the distance between them grew, suggesting that drying temperature is the most important factor affecting their lipid composition. Overall, the temperature of 50 °C was selected the ideal

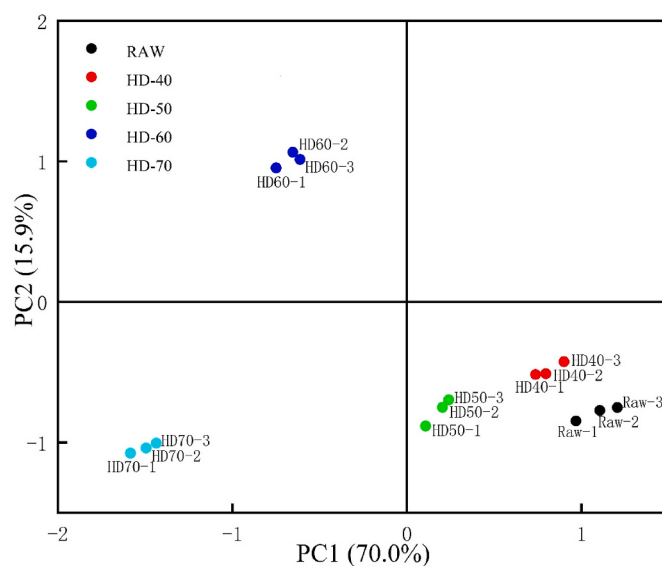


Fig. 3. Score scatter plot of PCA. Samples of RAW, HD-40, HD-50, HD-60 and HD-70 represent Tai Lake whitebait sampled as raw fresh, and heat dried at 40, 50, 60 and 70 °C.

temperature for drying.

4. Conclusion

In conclusion, the impact of temperature on the quality characteristics and lipid oxidation of Tai Lake whitebait during the hot-air drying process was investigated. The results emphasize the importance of optimizing the drying process of Tai Lake whitebait to minimize lipid oxidation and preserve its quality characteristics. The study results suggest that higher drying temperatures have a significant impact on the deterioration of whitebait quality attributes and increased lipid oxidation. Specifically, elevated temperatures were associated with higher levels of POV, TBARS, AV, and TOTOX, indicating a degradation in the lipids. Furthermore, the drying temperature influenced the fatty acid composition, lipid composition, and phospholipid content of the whitebait. These results highlight the significance of optimizing the drying process of Tai Lake whitebait to reduce lipid oxidation and maintain its quality. By selecting the appropriate drying temperature of 50 °C, it is possible to enhance the overall quality of whitebait. The insights gained from this research can provide valuable guidance for the industry in developing improved drying strategies and ensuring the production of high-quality Tai Lake whitebait. Further investigations should explore additional factors affecting the drying process and assess their impact on the quality attributes of whitebait.

CRediT authorship contribution statement

Mingfeng Xu: Funding acquisition, Investigation, Methodology, Writing – original draft. **Qiwei Liu:** Methodology, Investigation, Validation. **Xiangxiang Ni:** Investigation, Formal analysis. **Chengcheng Chen:** Data curation, Formal analysis. **Xiaohong Deng:** Investigation, Methodology. **Yajie Fang:** Data curation, Formal analysis. **Xiu Wang:** Project administration, Funding acquisition, Resources. **Qing Shen:** Supervision, Writing – review & editing. **Rongrong Yu:** Conceptualization, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2024.115942>.

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