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# HPLC-QTRAP-MS-based metabolomics approach investigates the formation mechanisms of meat quality and flavor of Beijing You chicken

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ABSTRACT

Chicken meat quality and flavor are determined by abundant metabolites. In this study, HPLC-QTRAP-MS-based metabolomic analysis was used to evaluate the characteristic metabolites in the breast muscle of Beijing You chickens aged 56, 98, and 120 days. A total of 544 metabolites in 32 categories were identified, among which amino acids and organic acids were the most abundant. 60 and 55 differential metabolites were identified between 56 and 98 days of age, 98 and 120 days of age, respectively. The content of L-carnitine, L-methionine and 3-hydroxybutyrate increased significantly at 98 or 120 days of age. Arginine biosynthesis, purine metabolism, alanine, aspartic acid, and glutamic acid metabolism were important metabolic pathways that affect chicken meat flavor. This study can help to elucidate the metabolic mechanism of breast muscle during Beijing You chicken development and provide a theoretical reference for the improvement of chicken meat quality and flavor.

## 1. Introduction

Chicken meat production is the most efficient, making it one of the most popular meat products worldwide. Poultry meat is expected to account for 27 % of the total meat consumption in 2021, and the world's poultry meat production will reach 181 million tons by 2050 (Magdelaine, Spiess, & Valceschini, 2008). However, while the growth rate of broilers has been continuously improved genetically, the quality and flavor of chicken meat has been declining. Compared with yellow-feathered broiler, white-feathered broiler breeds show lower inosine 5′-Monophosphate (IMP) concentration, lower shear force and higher cooking loss (Qi, Liu, Zhou, & Xu, 2017). In addition, the occurrence of white striping and wooden breast has recently increased in white-feathered broiler breeds (Anthony, 1998). Beijing You chickens is a precious chicken breed in China with superior meat quality, which is rich in free amino acids, intramuscular fat, fatty acids, and other flavor substances (Zhao, Cui, Liu, Zheng, Chen, & Wen, 2011). Therefore, Beijing You chickens can be used as an important research material to study the mechanism of meat quality and flavor formation.

In recent years, many studies have confirmed that the quality and flavor of meat has become important, and consumers are paying more attention to it. Subbaraj, Kim, Fraser, and Farouk (2016) found that the content of compounds with antioxidant properties, such as L-methionine, sugar phosphate and guanosine, was higher in meat with stable color. In addition, many metabolites are closely related to the flavor of meat, which can be divided into two classes based on their solubility: water-soluble and lipid-soluble. The water-soluble precursor substances mainly include amino acids, carbohydrates, nucleotides and vitamins. Free amino acids such as glutamic acid and serine determine the flavor and taste of meat and can be used as quality indicators to evaluate the taste of meat (Leggio, Belsito, De Marco, Liguori, Siciliano, & Spinella, 2012). The composition of inosine 5′-Monophosphate affects the umami flavor and freshness of meat. Lipid soluble precursor substances are mainly lipid metabolites. After heat treatment, fatty acids can be transformed into volatile flavor substances such as aldehydes, ketones and alcohols, and then further enhance the meat flavor (Sun et al., 2022).

Metabolomics provides the qualitative and quantitative analysis of

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all metabolites in biological systems, which focuses on determining the relationship between metabolites and physiological and pathological changes (Ott, Aranibar, Singh, & Stockton, 2003). The two most commonly utilized analysis techniques in metabolomics are mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR), which have been widely used in meat quality analysis. Wang, Fang, He, Dai, and Fang (2017) used 600-MHz $H^1$ NMR to analyze the quality of duck meat, and clarified the difference of breast meat metabolism between Linwu ducks and Pekin ducks. Welzenbach et al. (2016) found that sphingolipid metabolism and glycolysis had a significant effect on drip loss by chromatography-mass spectrometry (GC–MS) and liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF/MS).

The age of animal has certain effect on meat quality and flavor. Wideman, O"Bryan, and Crandall (2016) reported that the meat of older animals had higher shear force and darker meat color. Katemala, Molee, Thumanu, and Yongsawatdigul (2021) found that the content of IMP in breast muscle of Korat hybrid chicken at 10–20 weeks of age was significantly lower than that at 8 weeks of age, and the content of polyunsaturated fatty acid (PUFA) was significantly decreased at 10–20 weeks of age. Using $H^1$ NMR, Xiao, Ge, Zhou, Zhang, and Liao (2019) found the content of lactate, creatine, IMP, glucose, carnosine, anserine, taurine and glutamine of Wuding chicken meat at 230 days of age was significantly different from that of other days of age. However, the relationship between metabolite changes and the formation of meat quality and flavor during chicken development has not been elucidated.

The objective of this study was to investigate the metabolic mechanism of meat quality and flavor during the development of Beijing You chickens by LC-MS/LC-based metabolomic approaches coupled with multivariate data analysis, and to provide a theoretical reference for the breeding work of broiler meat quality and flavor.

## 2. Materials and methods

### 2.1. Ethics statement

Animal welfare practices and experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006). All procedures were approved by the Animal Ethics Committee of Beijing University of Agriculture.

### 2.2. Animals and sample collection

A total of 150 1-day-old female Beijing You chickens used in this study were reared at the Wei farm in Beijing, China, under identical environmental and nutritional conditions. All chickens entered the experiment at the same time and were kept under the same conditions. Food and water were provided ad libitum, and the basal diet composition was shown in Table S1. Ten chickens were randomly selected at 56 (rapid growth stage), 98 (marketing stage) and 120 days of age (stage with high deposition of intramuscular fat), respectively. Breast muscles were rapidly sampled, snap frozen in liquid nitrogen, and stored at −80 ℃ for metabolomics analysis. The remained samples were kept at 4 ℃ for meat quality analysis.

### 2.3. Meat quality analysis

Live body weight was determined after 12 h of starvation at 56, 98 and 120 days of age. Meat color, pH and shear force were determined 24 h after slaughter according to the standard procedure (NY-T/1333–2007; Ministry of Agriculture of the People's Republic of China 2007). pH of meat was measured using digital pH meter (Hanna Instruments HI98163, Limena, Italy). The pH meter was inserted into the samples, and each position was measured three times. Meat color was measured on the surface of breast muscle using CR520 chroma meter (Konica Minolta Holdings, Inc., Tokyo Japan) and assessed according to the Commission International de l'Eclairage (CIE) system (L *, lightness; a *, redness; and b *, yellowness) (Zhang et al., 2009). Shear force was measured using C-LM3B tenderness meter (TENOVO International Co, ltd., Beijing, China). The cooked samples were cut into strips (1 cm × 1 cm × 1 cm) parallel to the direction of the myofibers and the shear force was detected. All meat quality were measured three times at different locations of the breast muscle.

### 2.4. Metabolite extraction

A total of 30 samples (50 mg each) from three developmental stages were used for metabolomic analysis. Each sample with 1000 μL of ice-cold methanol/water (70 %, v/v) were homogenized. Sterile steel balls precooled at −20 ℃ for 2 min were added to the mixture and homogenized at 30 Hz for 3 min by grinder (MM400, Retsch, Germany). The mixture was incubated for 1 min and then centrifuged at 12,000 rpm for 10 min at 4 ℃. The collected supernatant was used for the LC-MS/MS analysis.

### 2.5. Mass spectrometry

The sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC, Shim-pack UFLC SHIMADZU CBM A system, https://www.shimadzu.com/; MS, QTRAP®6500 + System, https://sciex.com/) equipped with an ESI Turbo Ionspray interface controlled by Analyst 1.6.3 564 software (ABSciex).

LC analysis conditions were as follows: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm × 100 mm); solvent A was water containing 0.04 % acetic acid (v/v), solvent B was acetonitrile containing 0.04 % acetic acid; gradient program, 95:5 V/V at 0 min, 5:95 V/V at 11.0 min, 5:95 V/V at 12.0 min, 95:5 V/V at 12.1 min, 95:5 V/V at 14.0 min.

The ESI source operation parameters were as follows: source temperature, 500 ℃; ion spray voltage, 5500 V (positive), −4500 V (negative); ion source gas I, gas II, and curtain gas were set at 55, 60, and 25.0 psi, respectively; the collision gas was high. For instrument tuning and mass calibration, 10 and 100 μmol/L polypropylene glycol solutions were used in QQQ and LIT modes, respectively. According to the eluted metabolites, a specific set of MRM transitions were monitored for each period.

During analysis, a quality control (QC) samples were injected at regular intervals (every 10 samples) throughout the analytical run, in order to ensure the stability of the system.

### 2.6. Qualitative and quantitative analysis of metabolites

Based on the self-built target standard malware database (MWDB) (Metware Biotechnology Co., ltd., Wuhan, China), metabolites were identified by the retention time and the secondary spectrum data according to the standards hosted on the database curated by Metware Biotechnology Co., ltd.

Metabolites were quantified by multiple reaction monitoring (MRM) analysis using triple quadrupole mass spectrometry. The precursor ions of the target substance were screened. The characteristic fragment ions were selected by QQQ filtration. MultiQuant software was used to integrate and correct the chromatographic peaks. The peak area of each chromatographic peak represented the relative content of the corresponding substance.

### 2.7. Statistical analysis

SPSS 22.0 software was used for statistical analysis of meat quality data. One way ANOVA and Duncan's multiple comparison were performed. $P < 0.05$ was used as the standard for significant differences.

Analyst 1.6.3 was used to process the mass spectrum data. Based on the local metabolic database, qualitative and quantitative analyses of the metabolites were carried out. Principal component analysis (PCA) was performed by the "prcomp" statistics function within R (V4.1.0). Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed using SIMCA-14.1. Logarithmic transformation (log2) was performed on the MS data before OPLS-DA, and the mean was centered. OPLS-DA was validated by permutation tests. The values for $R^2$ (cumulative interpretation ability of model) and $Q^2$ (predictive ability of model) were employed as initial indicators for evaluating the goodness of fit. Variable importance of projection (VIP) values of the metabolites were obtained from the OPLS-DA model. Metabolites with VIP values $\geq 1$ and fold change (FC) $\geq 1.5$, or FC $\leq 0.67$, were used as the screening criteria for differential metabolites. A heatmap was created using the "pheatmap" package in R. Multivariate analysis was performed using MetaboAnalyst 4.0 (https://www.metaboanalyst.ca/), including different stages of development on metabolic pathways and metabolite set enrichment.

## 3. Results and discussion

### 3.1. Determination of meat quality during the developmental process

We measured live body weight, meat color, pH and shear force of Beijing You chicken at different stages (Table 1). The live body weight of Beijing You chicken increased significantly with age ($P < 0.05$). The pH (5.67) of breast muscle at 98 days of age was significantly higher than that at 56 and 120 days of age. The shear force of breast muscle at 98 days of age was significantly lower than that at 120 days of age ($P < 0.05$), indicating higher tenderness at 98 days of age. The conclusion of Katemala et al. (2021) was consistent with our results that shear force of Korat hybrid chicken thigh meat increased with the extended feeding time. L* and a* of breast muscle had significant differences in different developmental stages of Beijing You chicken ($P < 0.05$). b* at 56 days of age was significantly lower than that at the other two stages ($P < 0.05$). The results of Li et al. (2019) showed that the b* of breast muscle gradually decreased with the age of Da-Heng meat type birds. This difference may be due to different breeds.

### 3.2. Metabolic profiles in breast muscle of Beijing You chicken during the developmental process

Through qualitative and quantitative analysis of the metabolites in the breast muscle of Beijing You chickens at 56, 98, and 120 days, 544 metabolites were identified (Fig. 1A) (Table S2), including 32 categories of metabolites, such as amino acids and their derivatives, organic acids and their derivatives, carbohydrates, lipids, nucleotides, vitamins, and alcohols (Table S3). Amino acids, organic acids, and carbohydrates were the three most abundant metabolites.

PCA of all metabolite data revealed that the metabolites in the breast muscle of Beijing You chickens changed significantly at different developmental stages (Fig. 1B). The results of the cluster analysis showed the relative abundance of metabolites among different developmental stages (Fig. 1C).

To investigate the differences between groups, OPLS-DA was used to evaluate metabolites in breast muscles. We observed clear separation between each two groups, indicating that the metabolic profiles of breast muscle at different stages were significantly different from each other (Fig. S1). At 56 and 98 days of age, the $R^2X$, $R^2Y$ and $Q^2$ values of OPLS analysis were 0.125, 0.913 and 0.694. At 98 and 120 days of age, the $R^2X$, $R^2Y$ and $Q^2$ values of OPLS analysis were 0.112, 0.862 and 0.596. The $Q^2$ value of the two OPLS-DA model was greater than 50 %, which has good predictive ability (Fig. S2). The corresponding permutation tests in OPLS-DA model were conducted to assess model fitting by iteration 200 times (Fig. S3). The p values of Perm $Q^2$ and Perm $R^2Y$ were $<0.005$, suggesting that there was no over-fitting in the models.

**Table 1**
Characteristics of breast muscle during development of Beijing You chicken.

| Traits | 56Day | 98Day | 120Day |
|---|---|---|---|
| Live body weight (g) | $515.63 \pm 19.46^c$ | $1062.91 \pm 29.77^b$ | $1162.49 \pm 18.52^a$ |
| pH | $5.67 \pm 0.10^b$ | $5.79 \pm 0.07^a$ | $5.67 \pm 0.10^b$ |
| Shear force (N) | - | $2.14 \pm 0.28^a$ | $3.53 \pm 0.81^a$ |
| Color parameters | | | |
| L* | $58.00 \pm 2.19^a$ | $50.61 \pm 1.49^c$ | $53.04 \pm 1.22^b$ |
| a* | $2.36 \pm 0.31^b$ | $1.64 \pm 0.47^c$ | $2.68 \pm 0.67^a$ |
| b* | $7.01 \pm 1.08^b$ | $11.38 \pm 0.81^a$ | $11.67 \pm 1.24^a$ |

One way ANOVA and Duncan's multiple comparison were performed.
All values are presented as means ± SD.
Values with different letters within a row are significantly different ($P < 0.05$).

#### 3.2.1. Amino acids

Amino acids were the most abundant metabolites. A total of 86 amino acids and their derivatives were observed (Fig. 2A). We found that the most abundant amino acid derivative was L-carnosine, which increased with age (Table S4). Xiao, et al. (2019) found that L-carnosine in breast muscle of Wuding chicken increased significantly at 110–200 days of age, which was similar with our results. L-carnosine is a natural dipeptide composed of β-alanine and L-histidine and is mainly present in the skeletal muscle (Kohen, Yamamoto, Cundy, & Ames, 1988). L-Carnosine is a potential active oxygen scavenger with antioxidant capacity, which can effectively inhibit fat oxidation and formation of high-iron myoglobin, thereby preserving meat color (D'Astous-Pagé et al., 2017). The redness (a*) of breast muscle of Beijing You chickens increased significantly with the increase of age (Table 1), which was consistent with that of L-carnosine content. This may provide further evidence that L-carnosine has a certain regulatory effect on meat color. Previous results showed that age had no significant effect on the L-carnosine content in the breast muscle of an indigenous Korean chicken breed (Jayasena et al., 2014), which was different from our results, probably due to the different breeds.

The levels of 22 amino acids and their derivatives, including glutathione and cystine, increased with age (Table S5). Glutathione is a tripeptide found in the highest concentrations in muscle tissue and it is critical for muscle metabolism and homeostasis (Baldelli, Ciccarone, Limongi, Checconi, Palamara, & Ciriolo, 2019). In addition, sulfur-containing glutathione may help improve meat flavor (Ueda, Yonemitsu, Tsubuku, Sakaguchi, & Miyajima, 1997). Cystine, a sulfur-containing amino acid, produces a sweet, meat-like flavor. The reaction of sulfur-containing amino acids with sugars leads to the formation of many sulfur-containing flavor compounds, which have a lower odor threshold and can easily affect meat taste (Zhou, Grant, Goldberg, Ryland, & Aliani, 2019). Meanwhile, the contents of 23 amino acids and their derivatives, including serine, alanine, and glutamic acid, decreased with age (Table S6). Deng, Xing, Li, Xu, and Zhou (2022) also found that flavor amino acids such as glutamic acid in Wens Yellow-Feathered Mahuang chicken of different ages showed the same trend as our results. These amino acids are important flavor precursors in meat. Glutamic acid is responsible for the umami flavor, and serine and alanine provide sweetness. However, the relationship between the above metabolite levels and meat quality requires further study.

#### 3.2.2. Organic acids

In our study, organic acids were the second most abundant metabolite. 115 organic acids and their derivatives were detected (Fig. 2B). Betaine and creatine were the top ten metabolites of organic acids (Table S4). The betaine content was achieved the maximum at 120 days of age. Betaine supplementation can increase the amino acid content in breast muscle to further enhance meat flavor (Chen, Wen, Cheng, Chen, Zhuang, & Zhou, 2019). The rapid accumulation of creatine at 98 days of age was followed by a rapid decline at 120 days of age. Previous results

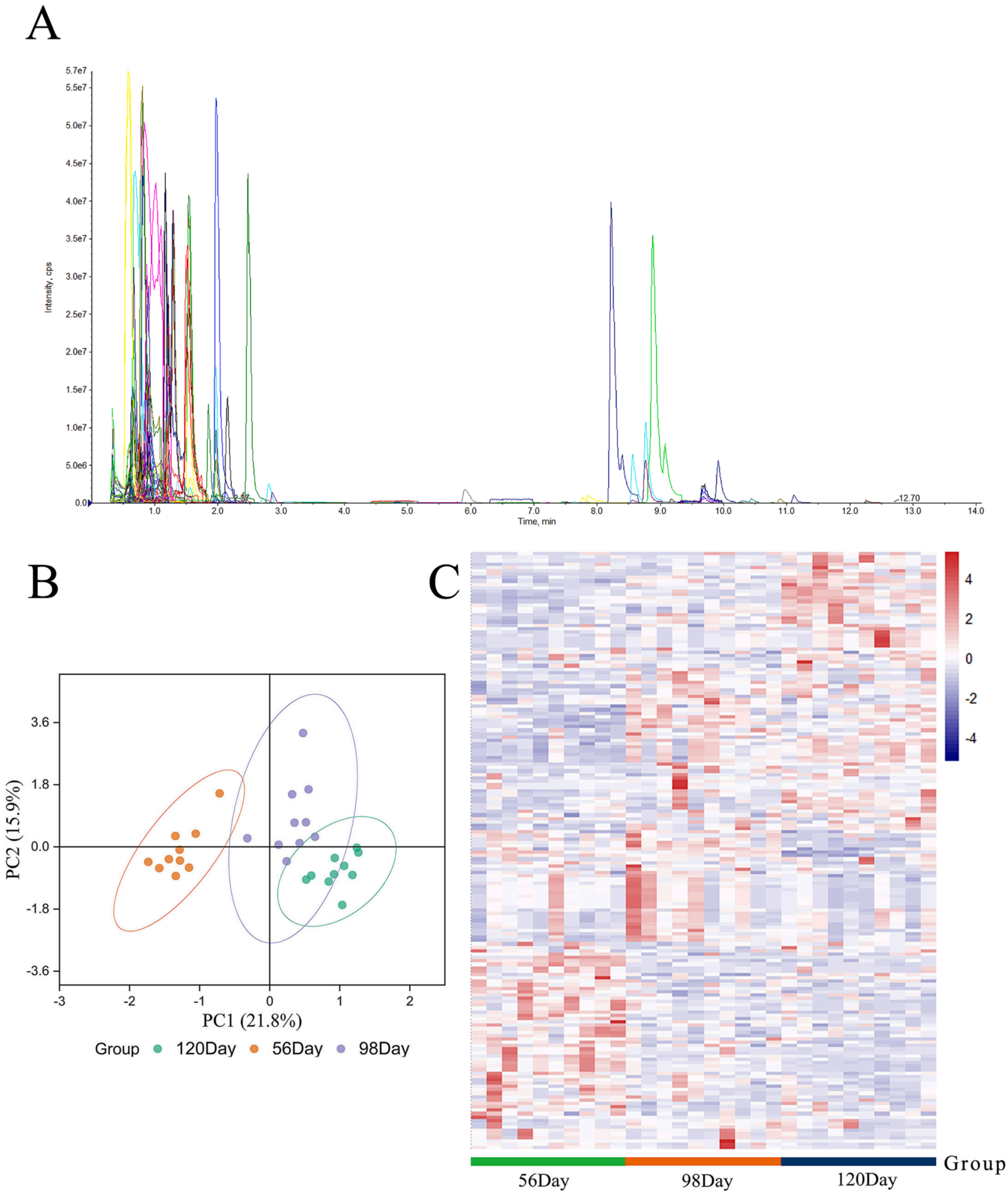


**Fig. 1.** (A) Multi-peak diagram of MRM metabolites detection. (B) Principal component analysis of metabolomics data from breast muscle of Beijing You chicken at three growth stages. (C) Heatmap of identified metabolites in breast muscle samples of Beijing You chicken at three growth stages. Red squares indicate increase of the relative amount of metabolites, and blue squares indicate decrease of the relative amount of metabolites. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

showed that creatine content was found to reach the highest level at 200 days of age in the breast muscle of Wuding chickens, which was different from our results, possibly due to differences in breed (Xiao, et al., 2019). Creatine is pivotal in muscle energy metabolism and muscle function. Increased creatine content may delay post-mortem lactate formation and postpone the pH decline in chickens (Nissen & Young, 2006). At 98 days of age, the pH was higher than that at 120 days of age (Table 1), which may be due to the accumulation of creatine at 98 days of age.

Lactic acid content was the highest at 120 days of age. The accumulation of lactic acid during growth can promote the decrease of muscle pH. In this research, the pH value decreased significantly at 120 days of age, indicating that lactic acid may have a certain impact on pH

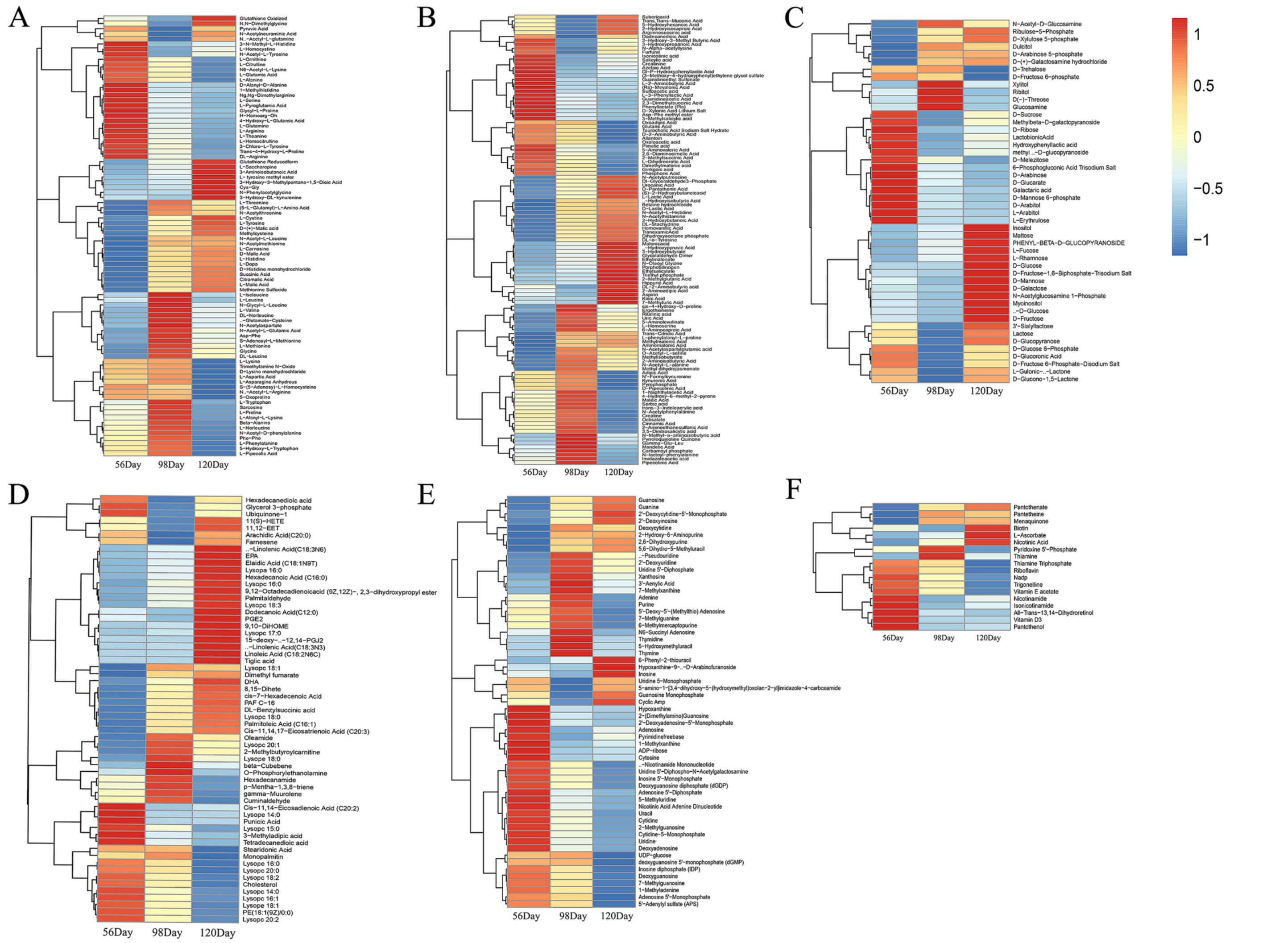


**Fig. 2.** Heatmap of classified metabolites in breast muscle of Beijing You chicken at three growth stages. Each sample or metabolite is represented by a single column or row, respectively. Red squares indicate increase of the relative amount of metabolites, and blue squares indicate decrease of the relative amount of metabolites. (A) Amino acids, (B) organic acids, (C) carbohydrates, (D) lipids, (E) nucleotides, (F) and vitamin and co-enzyme factor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of breast muscle. Compared with other phases, taurine content was higher at 98 days of age. In Wuding chicken, the content of taurine was highest at 140 days, which perhaps due to the difference in breed (Xiao, et al., 2019). Previous study has reported that taurine can significantly reduce drip loss, cooking loss and shear force of chicken, and increase the pH of breast muscle (Xu et al., 2020). Therefore, taurine may have some effect on improving the meat quality of breast muscle of Beijing You chicken.

### 3.2.3. *Carbohydrates*

In this study, 48 carbohydrates and their derivatives were detected (Fig. 2C). The top ten carbohydrate metabolites included two reducing sugars, L-fructose and D-galactose (Table S4). The Maillard reaction occurs between reducing sugars and amino acids during cooking, resulting in a series of volatile aromatic substances, which play an important role in meat flavor (Mottram & Nobrega, 2002). The rapid accumulation of L-fructose and D-galactose may further enhance the flavor of meat. The content of other reducing sugars, such as glucose, lactose, maltose, L-rhamnose and L-fucose, were the highest at 120 days of age. Furthermore, the addition of reducing sugars can induce protein glycation and facilitate the solubilization of myosin, which in turn might increase the water holding capacity of meat (Roldan, Loebner, Degen, Henle, Antequera, & Ruiz-Carrascal, 2015).

### 3.2.4. *Lipids*

61 lipids were detected in this study, including lipids, fatty acids, phospholipids, and oxidized lipids (Fig. 2D). The top ten lipid metabolites included Lysopc 18:1, Lysopc 18:2, Dimethyl fumarate, Lysopc 16:0, and so on (Table S4). Phospholipids are the main source of volatile flavors (Mottram, 1998). We found that the level of Lysopc 18:1, Lysopc 18:2, Lysopc 16:0 increased with age, which was beneficial to the formation of superior flavor.

Fatty acids are also important flavor precursors in chickens. The degradation products of fatty acids containing unsaturated bonds after heating participate in the Maillard reaction and can produce the characteristic aroma of fat and meat (Fan et al., 2018). In this study, rapid increases in the levels of γ-linolenic acid (C18:3), lauric acid, α-linolenic acid, docosahexaenoic acid, and palmitoleic acid were found at 120 days, which can improve the meat flavor and are positive for human health. The accumulation of these substances provides a basis for the formation of meat flavor.

### 3.2.5. *Nucleotides, vitamin and co-enzyme factor*

59 nucleotides were detected in this study (Fig. 2E). Nucleotides may

affect the meat quality. IMP, inosine, and hypoxanthine-9-β-D-arabinosine were the most abundant metabolites of nucleotides (Table S4). IMP and guanosine 5́-monophosphate (GMP) are important indicators for measuring the umami flavor of meat (Stanska & Krzeski, 2016). We found that GMP had the highest content at 120 days of age, but the highest content of IMP was at 56 days of age. IMP is an intermediate in the purine synthesis pathway (Srinivasan, Torres, & Ribas de Pouplana, 2021) and it is speculated that its decline may be caused by the synthesis of other functional nucleotides such as inosine.

18 vitamin and coenzyme factors were detected in this study (Fig. 2F). The most abundant vitamin metabolites were isonicotinamide and nicotinamide (Table S4). Our results showed that isonicotinamide and nicotinamide content were lowest at 98 days of age, and increased slightly at 120 days of age. Wang et al. (2020) found that the content of nicotinamide in the breast muscle of Linwu duck increased significantly with age. Isoniacinamide has a similar structure to niacinamide, which is the main form of vitamin B3 (niacin) in animals. Nicotinamide in breast meat is negatively correlated with cooking loss, and has the effect of increasing the content of unsaturated fatty acids in breast muscle and improving muscle quality (Wang, et al., 2020). In addition, the content of thiamine reached a maximum at 98 days of age. Thiamine is a bicyclic compound containing sulfur and nitrogen, which are the main water-soluble flavor precursors (Mottram, 1998). It can produce meat flavor substances through the Maillard reaction and degradation of thiamine.

### 3.3. Differential metabolites during Beijing You chicken development

To investigate the significant changes in metabolites among different growth stages, differential metabolite analyses were performed between 56 and 98 days of age, and between 98 and 120 days of age. The results showed that 31 metabolites were upregulated and 29 were down-regulated at 98 days of age compared to 56 days of age (Table S7)

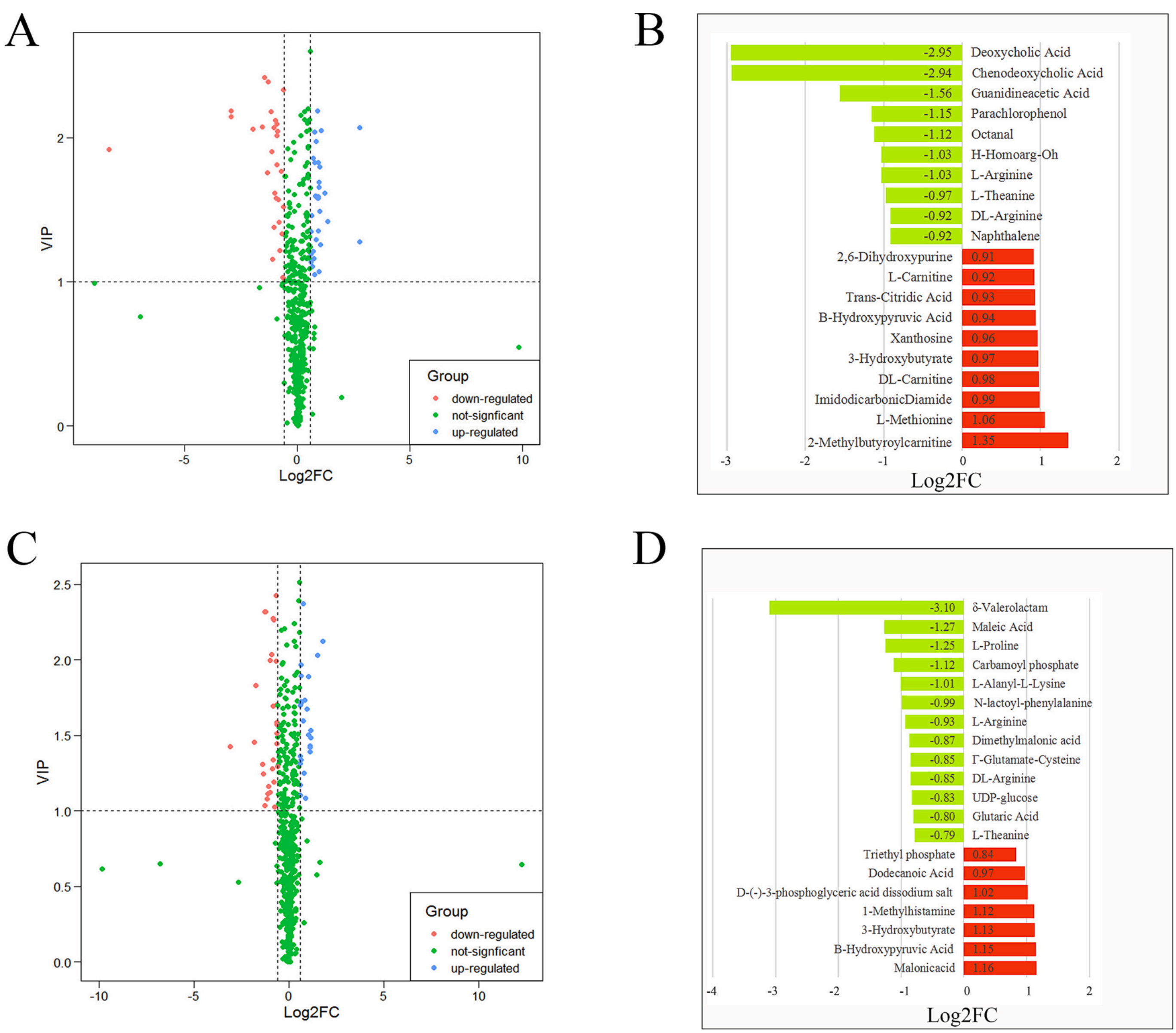


**Fig. 3.** Differentially accumulated metabolites in breast muscle of Beijing You chicken between different growth stages. (A) Volcano plots of differential metabolites between 56 days and 98 days of age, and 98 days vs 120 days. (B) Bar graph of the top 20 metabolites with $log_2FC$ between 56 days and 98 days of age. (C) Volcano plots of differential metabolites between 98 days and 120 days of age. (D) Bar graph of the top 20 differential metabolites with $log_2FC$ between 98 days and 120 days of age. Red squares indicate up-regulated differential metabolites and green squares indicate down-regulated differential metabolites. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Fig. 3A). Among them, the content of L-methionine at 98 days of age was 2.08 times higher than that at 56 days of age (Fig. 3B). L-methionine is a sulfur-containing amino acid. After heating, it produces dimethyl sulfide with a fragrant smell, forming an aroma of food. The content of DL-carnitine and L-carnitine was significantly increased at 98 days of age compared to that at 56 days of age. Previous studies have showed that in poultry production, L-carnitine promotes growth, enhances immunity, and improves antioxidant capacity (Ghoreyshi et al., 2019). In addition, L-carnitine can increase breast muscle production, reduce abdominal fat content, and increase intramuscular fat deposition in broilers (Xu, Wang, Mao, Zhan, & Hu, 2003). Compared with 56 days of age, the deoxycholic acid content decreased most significantly at 98 days of age. A previous study demonstrated that supplementing animals with deoxycholic acid impairs the homeostasis of glucose (Zaborska, Lee, Garribay, Cha, & Cummings, 2018). The decrease in deoxycholic acid levels in this study may ensure the stability of carbohydrate metabolites.

Compared with 98 days of age, 26 metabolites were significantly upregulated and 29 were downregulated at 120 days of age (Table S8) (Fig. 3C). Compared with that at 98 days of age, the content of 3-hydroxybutyrate and 1-Methylhistamine increased significantly at 120 days of age (Fig. 3D). In skeletal muscle, 3-hydroxybutyrate has potent anti-catabolic activity (Thomsen et al., 2018). As a precursor of histidine synthesis, 1-Methylhistamine plays a role in the synthesis of flavor substances such as imidazole dipeptides (carnosine and goose carnosine). L-theanine and L-proline significantly downregulated metabolites at 120 days of age compared to those at 98 days of age. L-proline has nutritional value in poultry growth. Drip loss and shear force decreased in response to dietary L-theanine supplementation (Zhang, Wang, Zhao, Chen, & Geng, 2020).

During the development of Beijing You chicken, the level of six metabolites were significantly increased, including L-rhamnose and L-fucose (Table 2). L-fucose and L-rhamnose not only have a certain sweetness, but also participate in the Maillard reaction to enhance meat flavor. In addition, the level of six metabolites were significantly decreased, including L-arginine and guanidine acetic acid (Table 2). Previous studies have reported that supplementation of animals with L-arginine or guanidine acetic acid can affect their water-holding capacity (Liu et al., 2015).

### 3.4. Metabolic pathway analysis

To further explore the most relevant pathways of chicken meat flavor, we analyzed all metabolites by KEGG pathway analysis. Fourteen pathways were predicted, of which the FDR values of 4 pathways were $<0.05$ (Table S9). Three metabolic pathways were closely related to chicken quality and flavor, including arginine biosynthesis, purine metabolism, alanine, aspartic acid, and glutamate metabolism (Fig. 4).

The arginine biosynthesis pathway was found to be the most significant. Studies have shown that dietary supplementation with arginine causes a series of physiological or chemical changes in chicken meat, such as affecting fat metabolism-related genes expression in skeletal muscle, promoting intramuscular fat deposition and reducing lightness (L*) and shear force of meat (Jiao, Guo, Yang, & Long, 2010).

Alanine, aspartic acid, glutamate metabolism, and purine metabolism are closely related to meat flavor. The umami flavor strongly associate with the presence of the free amino acids, aspartic acid and glutamic acid. Furthermore, nucleotides such as IMP and GMP have a synergistic effect with amino acids, boosting the umami flavor (Mouritsen & Khandelia, 2012). Therefore, arginine biosynthesis, purine metabolism, alanine, aspartic acid, and glutamate metabolism were the main metabolic pathways affecting the meat quality of Beijing You chickens.

## 4. Conclusions

The present analysis based on HPLC-QTRAP-MS provides novel insights into changes in metabolites in the breast muscle of developing Beijing You chickens. By combining metabonomics and multivariate statistical methods, we identified 544 metabolites and demonstrated that age is an important factor affecting the metabolites of chicken. Amino acids, organic acids, and carbohydrates were the three most abundant metabolites. The metabolites in the breast muscle of Beijing You chickens significantly changed during development. 60 and 55 differential metabolites were identified between 56 and 98 days of age, 98 and 120 days of age, respectively. The content of L-carnitine, L-methionine and 3-hydroxybutyrate increased significantly at 98 or 120 days of age. Furthermore, arginine biosynthesis, purine metabolism, alanine, aspartic acid, and glutamic acid metabolism were important metabolic pathways that affect the flavor of Beijing You chicken meat. These results can help to elucidate the metabolic mechanism of breast muscle during Beijing You chicken development, and provide a theoretical reference for improving the quality and flavor of chicken.

## CRediT authorship contribution statement

**Yu Ge:** Data curation, Writing – original draft. **Kai Gai:** Investigation, Resources. **Zheng Li:** Methodology, Data curation. **Yu Chen:** Funding acquisition, Supervision. **Liang Wang:** Funding acquisition, Supervision. **Xiaolong Qi:** Methodology, Data curation, Resources. **Kai Xing:** Methodology, Software. **Xiangguo Wang:** Investigation. **Longfei Xiao:** Investigation. **Hemin Ni:** Project administration. **Yong Guo:** Resources, Project administration, Supervision. **Li Chen:** Funding

**Table 2**
The differential metabolites in breast muscle of Beijing You chicken increased or decreased continuously during development.

| Item | Differential metabolites | 56Day | 98Day | 120Day |
|---|---|---|---|---|
| Up-regulated | B-Hydroxypyruvic Acid | $19.57 \pm 0.33^{c}$ | $20.67 \pm 0.26^{b}$ | $21.70 \pm 0.30^{a}$ |
| | L-Fucose | $12.85 \pm 0.36^{b}$ | $13.63 \pm 0.27^{b}$ | $14.62 \pm 0.29^{a}$ |
| | Malonicacid | $19.64 \pm 0.32^{c}$ | $20.69 \pm 0.25^{b}$ | $21.69 \pm 0.31^{a}$ |
| | L-Rhamnose | $12.05 \pm 0.27^{b}$ | $12.71 \pm 0.29^{b}$ | $13.75 \pm 0.33^{a}$ |
| | 3-Hydroxybutyrate | $19.45 \pm 0.32^{c}$ | $20.61 \pm 0.24^{b}$ | $21.55 \pm 0.32^{a}$ |
| | DHA | $11.32 \pm 0.18^{c}$ | $12.09 \pm 0.27^{b}$ | $12.79 \pm 0.19^{a}$ |
| Down-regulated | D-Glucarate | $14.49 \pm 0.14^{a}$ | $13.20 \pm 0.12^{b}$ | $12.51 \pm 0.15^{c}$ |
| | L-Theanine | $22.75 \pm 0.17^{a}$ | $21.84 \pm 0.10^{b}$ | $21.05 \pm 0.11^{c}$ |
| | Guanidineacetic Acid | $16.15 \pm 0.26^{a}$ | $14.23 \pm 0.36^{b}$ | $13.60 \pm 0.18^{b}$ |
| | L-Arginine | $20.98 \pm 0.18^{a}$ | $19.98 \pm 0.14^{b}$ | $19.03 \pm 0.18^{c}$ |
| | DL-Arginine | $25.15 \pm 0.18^{a}$ | $24.30 \pm 0.11^{b}$ | $23.45 \pm 0.12^{c}$ |
| | 5-Aminovaleric Acid | $16.12 \pm 0.30^{a}$ | $14.58 \pm 0.36^{b}$ | $13.23 \pm 0.20^{c}$ |

The values in the table represent the relative content of metabolites.
One way ANOVA and Duncan's multiple comparison were performed.
All values are presented as means ± SE.
Values with different letters within a row are significantly different ($P < 0.05$).

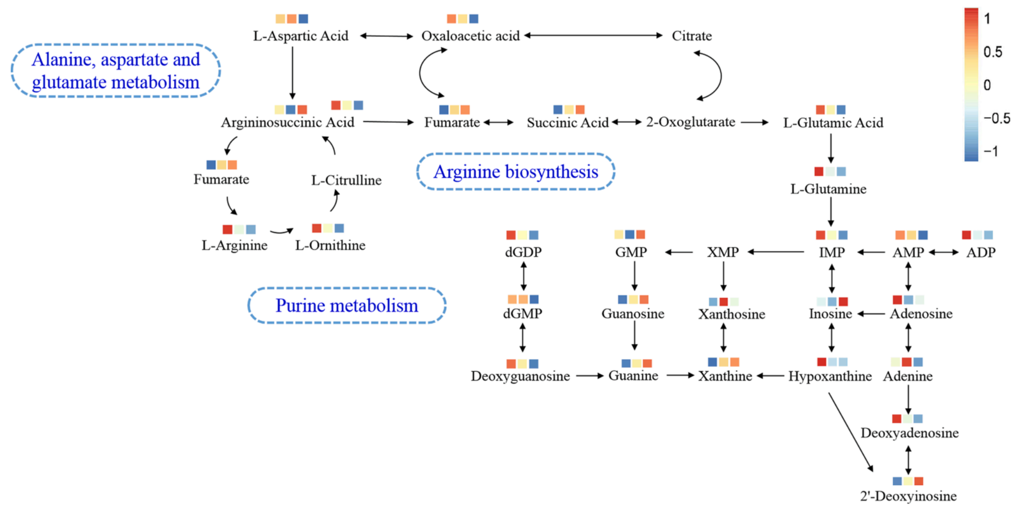


**Fig. 4.** The pathways related to meat quality and flavor during development in Beijing You chicken by KEGG pathway analysis.

acquisition, Methodology. **Xihui Sheng:** Conceptualization, Project administration, Funding acquisition, 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.fochx.2022.100550.

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