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Metabolomic comparison of meat quality and metabolites of geese breast muscle at different ages

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ARTICLE INFO	A B S T R A C T		
A R T I C L E I N F O Keywords: Yangzhou goose Meat quality Meat age Nontargeted metabolomics	The purpose of this study was to distinguish the effect of age on the meat quality and chemical composition of Yangzhou goose breast meat. Nontargeted metabolomics analysis (UHPLC-MS/MS) was used to distinguish the metabolic composition of goose meat at different ages, and Pearson's correlations between differential metabolites and key meat parameters were assessed. Compared with goslings, adult geese had lighter, redder and chewier meat ($p < 0.05$). Metabolite analysis revealed significant differences in nucleosides, organic acids, amino acids and sugars. Levels of IMP, xanthosine, pretyrosine and L-threonine were significantly higher in older meat ($p < 0.05$) and positively correlated with meat freshness indicators. However, pyruvic acid, L-cysteine and glucose 6-phosphate were up-regulated in gosling meat ($p < 0.05$), which were important flavor compounds. These results facilitate the further investigation of changes in goose meat composition and provide biomarkers for determining goose meat quality at different ages.		

Introduction

Meat products are important sources of protein in human diets and poultry meat provides eating attributes that fulfil expectations not normally achieved by other protein sources (Magdelaine et al., 2008). In recent years, the demand for poultry meat has risen rapidly worldwide and China is a major producer of goose meat (Razmaite et al., 2022). Goose meat has high nutritional value, is a particularly good source of amino acids and has specific aroma and flavor traits unlike other poultry (Okruszek et al., 2013). Meat quality is a complex trait that is affected by many factors including diet, genotype, age, and sex (Biesek et al., 2020). Age of birds has a great influence on the functional properties of chicken and meat tenderness tends to decrease with poultry age (Schneider et al., 2012). Furthermore, with increasing age, there is an increase in lipid content of duck breast and breasts became darker and redder (Baéza et al., 2000). Xiao et al. (2019) suggested that chicken meat at 230 days contained more glucose, inosine 5'-phosphate (IMP), anserine and glutamine than younger meat. In duck, compared with 50-day-old meat, 170- and 500-day-old meat was superior in regards to meat color, tenderness, and an appropriate meat to fat ratio, resulting in better taste (Liu et al., 2013). Weng et al. (2021a) suggested that 120-day-old geese had a larger muscle fiber area, higher intramuscular fat and elevated

protein content. However, detailed differences in meat quality between adult geese and goslings have not been specified.

Metabolomics is a widely employed omics techniques for studying food quality and food components. Gas chromatography mass spectrometry (GC–MS) and liquid chromatography MS (LC-MS) were used to assess goose meat quality (Fornal & Montowska, 2019) and ultra-highperformance LC tandem MS (UHPLC-MS/MS) was applied to examine metabolites changes in chilled chicken (Zhang et al., 2020). UHPLC offers high peak capacity, high resolution, sensitivity and high-speed analysis (de Villiers et al., 2006). Thus, combining UHPLC with tandem mass spectrometry (MS/MS) provides significant advantages including selectivity, sensitivity, and speed (Romero-González et al., 2008). UHPLC-MS/MS has been used to distinguish biomarkers of tropical fruits (Bataglion et al., 2015), fish muscle (Grande-Martinez et al., 2018) and egg yolk (Gao et al., 2021). However, metabonomic profiling of goose meat quality by UHPLC-MS/MS has not been reported.

Yangzhou goose, a major poultry species in Jiangsu Province, is renowned for high egg and good meat quality. The main purpose of the present study was to determine the effect of age on meat quality of Yangzhou goose and apply nontargeted metabolomics analysis (UHPLC-MS/MS) to determine metabolite profiles of geese at different ages. The results provide a theoretical basis for distinguishing differences in meat

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quality between young and adult Yangzhou geese, and provide biomarkers for distinguishing meat quality of geese at different ages.

Materials and methods

Ethics approval

This study was reviewed and approved by the Institutional Animal Care and Use Committee of the Department of Animal Science and Technology, Yangzhou University, China. All goose procedures were performed according to the Standards for the Administration of Experimental Practices (Jiangsu, China, 2008).

Goose rearing and sample preparation

Yangzhou geese were selected from the same commercial goose farm (Gaoyou, Yangzhou, China). Sixteen 70-day-old goslings (Youth) were randomly selected from a flock of 1000 goslings and 16 300-day-old healthy geese (Adulthood) were randomly selected from a flock of 300 adult geese, which were raised under a conventional method of stocking and supplementary feeding (Table S1). In addition to feed, geese were free to graze grass (Yu et al., 2020). Geese were maintained under natural daylight and temperature. Birds were stunned using a stun bath and exsanguinated by severing the jugular vein and carotid artery on one side of the neck. The left breast muscle of each goose was divided into two parts; one part was used to measure water-holding capacity, shear force, color, pH and for texture profile analysis (TPA) (Xiao et al., 2021); the other part was maintained in a sterile polythene bag and stored at 4 °C. Sterile breast muscle of geese was divided into two groups based on goose age (Y and A). There were eight replicates in each group with 50 g of breast muscle per sample.

Meat quality

Expressible moisture was determined using a meat quality pressure meter (Tenovo Meat-1, Beijing, China) and shear force was measured using a C-LM3B digital tenderness meter (Tenovo). The pH value was measured 45 min after slaughter using a pH-STAR pH meter (Matthaus, Berlin, Germany). Meat color was measured at three randomly selected positions using a CR-400 chroma meter (Konica Minolta, Osaka, Japan) and colorimetric parameters L*, a* and b* were recorded.

The TPA content of the goose breast muscle $(30 \times 30 \times 25 \text{ mm})$ was measured by a TMS-PRO texture analyzer (FTC, Sterling, USA) equipped with a 2500 N load cell. A double compression cycle test was performed, TPA analysis was performed using the following feature detection parameters: test speed, 20 mm/min; sample deformation, 20%; height of the load cell, 20 mm; trigger force, 30 N. TPA parameters (hardness, elasticity, cohesiveness, and chewiness) were calculated from force-time curves generated from samples using FTC-PRO software (FTC, Sterling, USA). All measurements were performed in triplicate.

Sample preparation for metabolomics analysis

Metabolite extraction was carried out as described by Dunn et al. (2011). A 25 mg sample of breast muscle was extracted by adding 800 μ L of precooled extraction reagent and internal standards mix 1 and 2 were added for quality control of sample preparation. After homogenizing for 5 min using a TissueLyser (JXFSTPRP, Shanghai, China), samples were sonicated for 10 min and incubated for 1 h at -20 °C. Samples were centrifuged for 15 min at 8000g and 4 °C and the supernatant was transferred for vacuum freeze-drying. Metabolites were resuspended in 200 μ L of 10% methanol and sonicated for 10 min at 4 °C. After centrifuging for 15 min at 8000×g, supernatants were transferred to an autosampler vials for LC-MS analysis. A quality control (QC) sample was prepared by pooling the same volume of each sample to evaluate the reproducibility of the whole LC-MS analysis.

LC-MS analysis

Samples were analyzed on a Waters 2D UPLC instrument (Waters Corporation, MA, USA) coupled to a Q-Exactive MS instrument (Thermo Fisher Scientific, MA, USA) with a heated electrospray ionization source and controlled by Xcalibur 2.3 software (Thermo Fisher Scientific, MA, USA). Chromatographic separation was performed on a Waters ACQ-UITY UPLC BEH C18 column (1.7 μ m, 2.1 mm \times 100 mm; Waters) and the column temperature was maintained at 45 °C. MS settings for positive/negative ionization modes were as follows: spray voltage, 3.8/-3.2 kV; sheath gas flow rate, 40 arbitrary units (arb); aux gas flow rate, 10 arb; aux gas heater temperature, 350 °C; capillary temperature, 320 °C. The full scan range was 70–1050 *m/z* with a resolution of 70,000 and an MS/MS resolution of 17,500. The stepped normalized collision energy was set to 20, 40 and 60 eV.

Quality control, compound detection and annotation

Data quality was assessed according to the repeatability of QC sample detection, which was based on the base peak chromatogram (BPC) of all QC samples. Each sample was selected for BPC chromatogram display. The BPC chart should have good peak shape and large peak capacity. The reliability and stability of instrument performance were evaluated using principal component analysis (PCA) of all samples. Raw MS data collected by LC-MS/MS were imported into Compound Discoverer 3.1 (Thermo Fisher Scientific, MA, USA) for data processing. Identification of metabolites was performed using BGI self-built standard library, mzCloud and ChemSpider (HMDB, KEGG, LipidMaps) databases. Parameters for metabolite identification were precursor mass tolerance < 5 ppm, fragment mass tolerance < 10 ppm, retention time tolerance < 0.2 min.

Differential metabolite analysis

Multivariate statistical analyses principal coordinate analysis (PCA) and partial least squares discriminant analysis (PLS-DA), univariate analysis, fold-change and Kruskal-Wallis test were combined to screen for differential metabolites between groups. PCA and PLS-DA were used to establish a relationship model between metabolite expression and sample groups and thereby predict the sample group, then combined with fold-change and t-tests to determine differential metabolites. Differential metabolite screening criteria were as follows: variable importance in the projection (VIP) of the first two principal components of the PLS-DA model \geq 1; fold-change \geq 1.2 or \leq 0.83; p < 0.05.

Statistical analysis

Data analyses were performed with SPSS 13.0 software (SPSS Inc., Chicago, USA). Results are expressed as mean \pm standard error, the statistical significance of differences among the various groups was evaluated by one-way analysis of variance in the GLM procedure and p < 0.05 was considered statistically significant.

Results

Meat quality

The meat quality of the breast muscle of geese at different ages is shown in Table 1. The cooking loss of 70-day-old goslings was 27.41%, which was very significantly higher than 300-day-old geese (p < 0.01), while the shear force was very significantly lower than that of adult geese (p < 0.01). The pH value of goslings was higher than that of adult geese (p < 0.05). Although the L* and b* breast muscle values were very significantly lower in adult geese than goslings (p < 0.01), the a* value showed no significant difference between the two groups (p > 0.05), suggesting adult geese had better meat color.

Table 1

Breast meat quality of geese at different ages.

Item	Adulthood	Youth
Cooking loss (%)	20.34 ± 4.58	$27.41 \pm 3.25^{**}$
Shear force (N)	$\textbf{86.04} \pm \textbf{14.91}$	$57.53 \pm 5.66^{**}$
pH value	6.31 ± 0.25	$6.59\pm0.14^*$
L*	30.35 ± 1.15	$50.21 \pm 5.56^{**}$
a*	14.62 ± 0.62	$12.55\pm3.06^{\rm ns}$
b*	$\textbf{4.47} \pm \textbf{0.51}$	$7.00 \pm 1.84^{**}$

^{*} Significant at P < 0.05.

^{**} Significant at P < 0.01.

^{ns} Not significant at P > 0.05.

TPA parameters

The textural properties of the breast muscles of geese at different ages are shown in Table 2. Results from TPA analysis showed that the hardness and cohesiveness of meat from geese at different ages showed no significant difference (p > 0.05). The springiness and gumminess of adult geese was higher than that of goslings (p < 0.05). The chewiness of adult geese was 9.93 mJ, very significantly higher than for goslings (p < 0.01), confirming that adult geese were chewier.

Metabolic profiles of goslings and adult geese

The BPCs of all QC samples overlapped suggesting perfect repeatability and the signal was stable during detection and analysis (Fig. 1). A total of 4330 and 2213 peaks were detected using positive and negative ion modes (Tables S1 and S2). The PCA score plot showed a low dispersion of QC samples (Fig. 2), based on positive and negative ion modes. Furthermore, PLS-DA ($R^2Y = 0.92$, $Q^2Y = 0.76$) was used to confirm significant differences in metabolic profiles, R^2 -values were lower than the original point and the negative intercept of Q^2 indicated the reliability of PLS-DA.

Identification of differential metabolites

LC-MS/MS-based nontargeted metabolomics was used to detect changes in metabolic profiles of geese at different ages. It was obvious that the metabolic profiles were different between goose meat at different ages. Hierarchical clustering and heatmap analyses were performed to assesses metabolites in geese at different ages (Figs. S2 and S3), 733 and 615 metabolites from A and Y groups were identified in positive and negative ion modes, respectively. Additionally, 35 differential metabolites were identified, including fatty acids, organic acids, amino acids and nucleosides (Table 3). Furthermore, compared with adult geese, 17 metabolites were increased and 18 metabolites were decreased in young geese. Thiamine, L-glutamine, L-cysteine and Lasparagine were significantly increased in young geese, while γ -linolenic acid, creatine, IMP, hypoxanthine and cinnamic acid were higher in adult geese.

Metabolic pathways of differential metabolites between goslings and adults

To explore reveal the pathways of differential metabolites in the breast muscle of geese at different ages, we performed enrichment

 Table 2

 TPA parameters for breast muscle of geese at different ages

1	0	0
Item	Adulthood	Youth
Hardness (N)	52.28 ± 5.22	53.78 ± 2.72^{ns}
Cohesiveness (%)	$0.55\pm0.0.08$	$0.57\pm0.02^{\rm ns}$
Springiness (mm)	0.28 ± 0.12	$0.15\pm0.02^{\ast}$
Gumminess (N)	37.36 ± 4.23	$33.42 \pm 1.81^{*}$
Chewiness (mJ)	9.93 ± 3.19	$5.26 \pm 0.82^{**}$

analysis based on the 35 important metabolites (Fig. 2). Of the 16 KEGG pathways, purine metabolism was the most enriched, which included seven metabolites. Compared with adult geese, inosine and guanosine monophosphate were significantly increased in young geese, by 2.92-fold and 1.6-fold, respectively, while L-threonine, IMP, hypoxanthine and xanthosine were significantly decreased in young geese. L-threonine was significantly increased in adult geese and was enriched in seven pathways including glycine, serine and threonine metabolism, valine, leucine and isoleucine biosynthesis, porphyrin and chlorophyll metabolism and biosynthesis of amino acids, suggesting L-threonine plays an important role in meat flavor of goose meat at different ages.

Analysis of key meat parameters and differential metabolites

Differential metabolites are key factors affecting the quality of goose meat at different ages. A total of 35 differential metabolites with high VIP values and eight important meat parameters were selected and correlations were assessed using the Pearson's method (Fig. 3). The results showed that L-glutamic acid, L-cysteine and thiamine had extremely strong positive correlations with L*, while pretyosine, xanthosine, gluconolactone and linamarin had extremely strong positive correlations with L*. Furthermore, pretyosine and xanthosine also had extremely strong positive correlations with chewiness, springiness, and shear force. However, L-cysteine had strong negative correlations with chewiness, springiness and shear force. Pyruvic acid and L-glutamic acid had strong positive correlations with cooking loss.

Discussion

Meat color is one of the most important fresh meat characteristics at the point of purchase (Gracia & de Magistris, 2013). In China, people prefer redder poultry meat (Guo et al., 2018). In our study, meat from 300-day-old geese was redder meat than that from 70-day-old geese. Moreover, older geese not only had redder meat (a*), but also had lighter (L*) and yellower (b*) meat. Li et al. (2019) reported similar results for chicken meat. Age is an important factor affecting meat color and texture since myoglobin levels increase with age (Lyon et al., 2004). Furthermore, a longer growth season results in chewier goose meat, which is popular in China (Weng et al., 2021a), and older geese produced chewier meat in the present study. Consistent with the results of Saláková et al. (2009), gosling meat had a higher muscle pH, which was associated with darker meat. Furthermore, higher shear force and lower cooking loss were observed in breast meat from older geese, similar to the results of Weng et al. (2021b). Li (2006) concluded that gumminess is a proxy of hardness and cohesiveness in hens, but in present study, gumminess and springiness were significantly higher in adult goose meat, and hardness and cohesiveness were not significantly lower in goslings, which may be related to differences in breeds and sex.

In this study, the results of metabolite analysis of breast meat of geese at different ages revealed significant differences in organic acids, nucleosides, sugars and amino acids. Organic acids, including pyruvic acid and γ -linoleic acid, have a strong influence on meat quality. Pyruvic acid is a cellular metabolite at a key biochemical junction of glycolysis (Maleki & Eiteman, 2017). In our study, pyruvic acid was elevated in gosling. Welzenbach et al. (2016) indicated that a high rate of glycolysis results in a high L* value, which was similar to that of the gosling meat in our study. Furthermore, pyruvic acid had a strong positive correlation with cooking loss in this study, and numerous studies concluded that a high glycolytic potential in muscles results in a high drip loss (Sieczkowska et al., 2010), indicating that pyruvic acid is an important biomarker related to meat quality of geese at different ages. Linoleic acid is the most highly consumed polyunsaturated fatty acid (PUFA) found in the human diet, and it can serve as both a source of energy and a structural component (Whelan & Fritsche, 2013). Linoleic acid was higher in adult geese than goslings in the present work, and della Malva et al. (2016) reported similar results in lamb meat. Conjugated linoleic



Fig. 1. Multivariate statistical analysis of goose meat at different ages based on UPLC-MS profiles. (A) Principal component analysis (PCA) score plot based on positive ion mode results; (B) PCA score plot based on negative ion mode results; (C) Permutation testing of the PLS-DA model with 200 repetitions based on positive ion mode results; (D) Permutation testing of the PLS-DA model with 200 repetitions based on negative ion mode results; (D) Permutation testing of the PLS-DA model with 200 repetitions based on negative ion mode results.

acid requirements in humans are mainly met by the consumption of animal-derived products, especially poultry products (Grashorn, 2007). Furthermore, it also showed positive correlations with chewiness and springiness, suggesting meat from adult geese can be consumed as a source of linoleic acid.

Nucleotides also affect meat flavor. IMP, xanthosine and hypoxanthine, involved in purine metabolism, were significantly elevated in adult geese. IMP plays a key role in the development of umami taste in chicken meat (Jung et al., 2013). It is hydrolyzed to hypoxanthine, which has a positive correlation with sweetness in cooked lamb (Bi et al., 2021). Furthermore, Huang et al. (2022) concluded that the IMP content of chicken muscle increased with increasing age, and meat quality was improved, consistent with better taste for adult geese in this study. Vani et al. (2006) found that phosphate hydrolysis from IMP to inosine was more rapid at lower pH, but adult geese had higher IMP levels and lower pH in the present study, and further research is needed to explore the transformation mechanism of IMP. Both xanthosine and inosine are indicators of meat freshness and play important roles in IMP metabolism (Fang et al., 2022). Xanthosine was up-regulated in adult geese and positively correlated with meat freshness indicators (chewiness, springiness, gumminess and shear force), while inosine was upregulated in gosling and was only weakly correlated with freshness indicators, suggesting that xanthosine can be used as a freshness indicator of goose meat.

Xiao et al. (2019) concluded that amino acids are the most abundant

metabolites affecting meat quality, and they are important flavor and flavor precursor substances in chicken meat. L-cysteine levels are higher in goslings and reaction of cysteine and sugars generates unique chicken flavor (Ames et al., 2001). Both cysteine and glucose 6-phosphate were elevated in goslings, and these two indicators had strong positive correlations with L*, indicating that they may affect the meat flavor and quality of goslings. In adult geese, both L-threonine and pretyrosine were elevated. L-threonine is sweet and can improve meat quality (Jiang et al., 2020), but it showed only a weak correlation with meat freshness indicators of goose meat. Pretyrosine is an obligatory intermediate of Ltyrosine biosynthesis, Leggio et al. (2012) found that L-tyrosine is closely related to meat flavor and can be used as an indicators of meat quality and freshness. Consistently, pretyrosine was strongly positively correlated with meat freshness in the present work.

Conclusion

In summary, this study demonstrated that springiness, gumminess, chewiness and shear force were significantly higher in 300-day-old geese, with lower L*, b* and higher a*, consistent with lighter, redder and chewier meat. The results suggest that age plays a vital role in the quality of goose meat. Furthermore, metabolites differed in geese between the ages of 70 and 300 days. Xanthosine was elevated in adult geese and positively correlated with meat freshness. Levels of pretyrosine and L-threonine were higher in adult geese and pretyrosine had a



Fig. 2. Changes in metabolites of goose meat at different ages. (A) Heatmap of differential metabolite; (B) Pathway prediction of differential metabolites based on KEGG analysis; (C) Pathway enrichment of differential metabolites.

Table 3

Differential metabolites of breast meat of geese at different ages.

Metabolite	Molecular weight	Retention time (s)	VIP	ESI	Direction (Y/A)
Docosahexaenoic acid	328.2392	9.881	1.3751**	pos	down
Thiamine	264.1044	0.755	1.5399**	pos	up
γ-Linolenic acid	278.2244	8.652	1.5**	pos	down
N-Acetyl-glucosamine 1-phosphate	301.0562	0.785	1.2221^{**}	neg	up
D-Glucose 1,6-bisphosphate	339.9961	0.586	1.9099**	neg	down
Sucrose 6-phosphate	422.083	0.898	1.4387**	pos	down
D-glucose 6-phosphate	260.0294	0.774	1.4317**	neg	up
Pretyrosine	227.0795	4.848	2.1477**	pos	down
Quinic acid	192.0643	0.84	1.8146**	pos	down
L-serine	105.0426	0.654	1.3871^{**}	neg	up
L-cysteine	121.0198	5.661	1.0934**	neg	up
Creatine	131.0695	0.701	1.3603^{**}	neg	down
L-threonine	119.0583	0.672	1.2095^{**}	neg	down
L-asparagine	132.0532	0.695	1.7922^{**}	pos	up
Inosine	268.0804	1.497	2.2946**	neg	up
Xanthosine	284.0756	2.842	2.0988 ^{**}	neg	down
Inosine 5'-phosphate	348.0471	0.717	1.9028^{**}	neg	down
Guanosine monophosphate	363.0579	0.706	1.6217^{**}	neg	up
3'-Adenosine monophosphate	347.0631	2.356	1.5655^{**}	pos	down
Hypoxanthine	136.0386	1.067	1.2616^{**}	neg	down
Adenine	135.0546	1.639	1.0263^{**}	neg	down
L-glutamine	146.0692	0.658	1.4908^{**}	neg	up
L-glutamic acid	147.0532	0.783	1.3842	neg	up
Trans-cinnamic acid	148.0525	2.672	1.3926^{**}	neg	down
Taurine	125.0147	0.648	1.3938^{**}	neg	up
Gluconolactone	178.0479	0.801	1.5903^{**}	pos	down
6-Phosphonoglucono-D-lactone	258.0141	0.596	1.5646^{**}	neg	down
Erythrose 4-phosphate	200.0086	0.609	1.5468^{**}	neg	down
D-sedoheptulose 7-phosphate	290.0401	0.773	1.1544^{**}	neg	up
Fumaric acid	116.011	0.604	1.9497**	neg	up
Citrate	192.0271	0.603	1.511**	neg	up
Oxoglutaric acid	146.0215	0.743	1.3612^{**}	neg	up
Pyruvic acid	88.0161	0.768	1.5394**	neg	up
Ferulic acid	194.0579	4.717	1.5437**	neg	up
Linamarin	247.1056	0.851	1.8836**	pos	down

VIP, variable importance in the projection; ESI, electrospray ionization; pos, positive ion mode; neg, negative ion mode; Y/A, youth/adulthood.



Fig. 3. Correlations between key meat parameters and differential metabolites of goose meat at different ages.

strong positive correlation with meat freshness. However, pyruvic acid was elevated in gosling meat and had a strong positive correlation with cooking loss. Cysteine and glucose 6-phosphate had strong positive correlations with L*, and they are important flavor compounds of gosling meat that can be used as biomarkers. The findings provide new insight into the molecular mechanisms underlying changes in metabolites in Yangzhou geese at different ages, and biomarkers for determining goose meat quality.

CRediT authorship contribution statement

Ying Wang: Conceptualization, Methodology, Validation, Writing – original draft. Wanqing Li: Methodology, Validation. Chi Zhang: Methodology, Data curation. Fushi Li: Methodology, Validation. Haiming Yang: Conceptualization, Writing – review & editing, Supervision, Funding acquisition. Zhiyue Wang: 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

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