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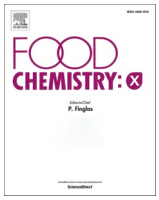

Insight into the chemical composition, antioxidant capacity, meat quality, fatty acid profile, and volatile compounds of yellow-feathered chickens fed with fermented pineapple residue

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ABSTRACT

This study aimed to evaluated the effect of dietary fermented pineapple residue (FPR) on the chemical composition, antioxidant capacity, meat quality, fatty acid profile, and volatile compounds in yellow-feathered chickens. GC-IMS technique combined with multivariate analysis were performed to clarify the key volatile compounds. The results showed that dietary FPR improved meat quality by increasing the antioxidant capacity and pH value and decreasing cooking loss of breast muscle. The fatty acid profile was altered in breast muscle of chickens that fed with FPR. GC-IMS detected 43 volatile compounds in breast muscle, including mainly aldehydes, alcohols, esters, and ketones. Among them, 12 volatile compounds could serve as potential aroma markers to distinguish meat flavor of chickens fed with FPR. Correlation analysis revealed that C18:1n9c, C18:2n6, and PUFA are important contributors for meat flavor formation. In conclusion, dietary FPR improved antioxidant capacity, meat quality, fatty acid profile, and volatile compounds of breast muscle in chickens.

1. Introduction

Chicken meat is very popular source of animal protein worldwide, due to its high-quality protein and low cholesterol. Especially, with the improvement of living standards, consumer demand for safer, healthier, and excellent-quality meat is increasing. In China, yellow-feathered chickens attract a large number of consumers due to the good taste profile of meat (Jin et al., 2021). However, chicken meat quality is compromised by environmental stress under the intensive poultry production (Bilal et al., 2021), which negatively affects consumers' purchasing decisions.

Meat quality characteristics include appearance, texture, juiciness, tenderness, flavor, and nutritional value. Aroma is a key index for evaluating meat flavor, more than 1000 volatile compounds have been identified and a large number of aldehydes, alcohols, esters, and ketones made great contributions to meat flavor (Kosowska, Majcher, & Fortuna, 2017). All these compounds are mainly oxidation products of fatty acids, especially unsaturated fatty acid (UFA), their composition plays a crucial role in flavor formation (Shahidi & Hossain, 2022).

Pineapple residue is a by-product of pineapple processing and accounts for approximately 30 % of the fresh pineapple (Selani et al., 2014). This residue could serve as potential antimicrobials, antioxidants, colorants, and flavorings, as they contain high levels of phenols, flavonoids, and carotenoids (Mirabella, Castellani, & Sala, 2014). Previous study has reported that pineapple residue supplementation can improve the taste profile of steer meat (Chaosap, Sahatsanon, Sitthigripong, Sawanon, & Setakul, 2021). Meanwhile, fermentation is a feasible way for bioconversion of fruit or plant residues. The fermentation process could improve the bioactive activities by metabolic activity of microorganisms (Aziz & Karboune, 2018). As is reported in the latest study, the fermentation was a effective way to increase the polyphenol content and antioxidant activity of rose residue through

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Lactiplantibacillus plantarum and *Bacillus subtilis* (Hu et al., 2022). Furthermore, a recent study found that supplementation with fermented tomato pomace improved levels of serum SOD and GSH-Px in broiler chickens (Gungor, Altop, & Erener, 2024). Dietary supplementation with fermented pineapple residue (FPR) had a positive impact on meat quality in Simmental bull (Deng, Liu, Li, Xu, & Zhou, 2022). However, whether dietary FPR can affect antioxidant capacity, meat quality, and volatile compounds in chickens are still unknown.

Gas chromatography-ion mobility spectroscopy (GC-IMS) has the power to rapidly identify volatile compounds by the high sensitivity and low detection limit (Wang, Chen, & Sun, 2020). Until now, it has been widely applied in meat science, for example, identifying the differences of volatile compounds during meat processing (Sun et al., 2023). Moreover, this technology has also been used in animal production to detect the volatile compounds in chicken breeds and slaughter ages (Deng, Liu, et al., 2022). However, there is a lack of information on the impact of nutritional strategy on volatile compounds in chickens by GC-IMS.

The agri-food industry produces large amounts of pineapple residue globally. Considering its properties, pineapple residue could be a promising additive in animal nutrition. Therefore, the objective of the current study was to explore the influence of pineapple residue on the chemical composition, antioxidant capacity, meat quality, fatty acid profile, and volatile compounds in yellow-feathered chickens by fermentation.

2. Materials and methods

All experimental procedures in this work received approval from the Animal Ethics Committee of South China Agricultural University (Permission number: 2023 g032).

2.1. Preparation of fermented pineapple residue

The FPR was purchased from Yinheng Biotechnology Co., Ltd. (Zhanjiang, Guangdong, China). Briefly, the pineapple peel was dried at room temperature for 6 days and ground by mill. *Lactiplantibacillus plantarum* (ATCC 8014) and *Bacillus subtilis* (ATCC 6051) were inoculated in the basal substrate including 70 % pineapple peel, 26 % wheat bran, 1 % $(NH_4)_2SO_4$, 1.60 % CO $(NH_2)_2$, 1 % KH_2PO_4, and 0.40 % $MgSO_4$. Afterward, the mixed substrate was fermented at 30 °C for 7 days, then the FPR was dried in an air circulatory tray drier at 45 °C for 3 days and ground for the experimental diet. The nutritional composition of FPR is presented in Supplementary Table 1. Herein, dry matter, crude protein, crude fat, crude fiber, and ash were determined according to the Association of Official Analytical Chemists (AOAC) method (AOAC, 2000). Total phenolic content was measured at 760 nm using commercial kit based on the Folin-Ciocalteu colorimetric method. Total flavonoid content was detected at 510 nm by commercial kit following the sodium nitrite-aluminum nitrate colorimetric method.

2.2. Experimental design and diets

Seven hundred and twenty 85-day-old female yellow-feathered chickens (Wens Co. Ltd., Yunfu, China) were randomly allocated to 4 groups. Each group contained 5 replicates with 36 birds each. The control group was provided a basal diet, and the other three groups received a basal diet containing 2 %, 4 %, and 8 % of FPR, respectively. All chickens received drinking water and feed *ad libitum* in the experimental room with a comfortable room temperature (25 °C) and 12 h of illumination throughout this experiment. The experiment lasted for 5 weeks. The basal diets were designed to meet the nutritional requirements of Chinese yellow feathered chicken (Supplementary Table 2).

2.3. Sample collection

At 120 days of age, 4 chickens with similar BW close to average BW of their replicate were selected and slaughtered via severing the jugular vein under chloroform anesthesia inhalation after fasting for 12 h. Breast muscles of 2 chickens from each replicate (10 chickens per group) were separated and collected to determine meat quality. Breast muscles of the other 10 chickens per group were collected and placed into liquid nitrogen immediately, then frozen at −80 °C until analysis.

2.4. Chemical composition

The chemical composition of breast muscle (moisture, crude protein, crude fat, and ash) was determined by the procedures of the AOAC (AOAC, 2000). The moisture was estimated by drying samples. The crude protein was measured by the Kjeldahl method. The crude fat was determined following Soxhlet extraction. The ash was analyzed by incineration in furnace at 550 °C.

2.5. Antioxidant capacity

Breast samples were homogenized and centrifuged at 3500 rpm at 4 °C for 15 min to obtain the supernatant. The total antioxidant capacity (T-AOC) was determined at 405 nm according to 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method. Glutathione peroxidase (GSH-Px) activity was determined by the consumption of GSH at 412 nm. WST-1 method was used to determined superoxide dismutase (SOD) activity at 450 nm. Malondialdehyde (MDA) was determined at 532 nm based on thiobarbituric acid (TBA) method. All parameters were detected using an Microplate Reader (Biotek, Synergy Neo2, USA) using the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. Meat quality determination

The physical properties in breast muscle were determined as follows: Meat pH analysis was performed at 3 random locations of breast muscle at 45 min using a pH meter (Testo 205, Germany). Meat color, including lightness (L^*), redness (a^*), and yellowness (b^*) was determined at 3 random locations of breast muscle using a chromameter (CR-410, Konica Minolta, Japan). Drip loss was determined by the slight modified method (Wei et al., 2022). Briefly, breast muscles strips (1 × 2 × 3 cm) were dissected and weighed, then were hung in plastic bags, and kept in refrigerator at 4 °C. The breast samples were reweighed after 24 h. Drip loss (%) was calculated by the initial and final weight from each sample. Cooking loss was determined according to the report by Yang et al. (2022). 20 g of breast muscles were placed in zipper bags and heated in a thermostat water bath with 75 °C of internal temperature, then cooled to room temperature. Cooking loss (%) was calculated between the initial and final weight of breast samples. Subsequently, the cooked samples were further measured for shear force. Briefly, breast meat strips (2 × 0.5 × 0.5 cm^3) were measured by a Tenderness Meter (C-LM3B, Harin, China).

2.7. Determination of fatty acid composition

The fatty acid composition of breast muscle was determined following previous study with some slight modifications (Li et al., 2021). The extracted lipids were hydrolyzed with chloroform-methanol. Fatty acid was converted to methyl ester by acetyl chloride, the internal standard was methyl undecanoate in the ester exchange reaction. The separation and quantification of fatty acid methyl esters (FAMEs) were carried out by using a gas chromatograph (7890B, Agilent technology, CA, USA) with a HP-88 fused capillary column (100 m × 0.25 mm × 0.2 μm, Agilent, USA). The flow rate of carrier gas (nitrogen gas) was 20.0 mL/min. The temperatures of injector and detector were 270 and

280 °C, respectively. Initially, the column temperature was set at 100 °C for 13 min, gradually increased by 10 °C/min to 180 °C for 10 min, and raised at 1 °C/min to 200 °C for 20 min, finally increased to 230 °C at 5 °C/min for 5 min. Fatty acid profile were analyzed by comparing the relative retention times of the peaks with the internal standard.

2.8. GC-IMS analysis

Volatile compounds in breast muscle were analyzed based on a GC-IMS instrument (G.A.S., Dortmund, Germany). Briefly, 2 g of breast sample was placed into a 20 mL headspace glass vial. After heating at 80 °C for 20 min, the sample (500 μL) was added into injector at 85 °C. Volatile compounds were isolated by an MXT-5 capillary column (15 m × 0.53 mm × 1 um, Restek, USA) at 60 °C. The carrier (nitrogen, 99.9 %) was programmed as follows: initially 2 mL/min for 2 min, increased to 20 mL/min for 8 min, and linearly increased to 100 mL/min for 10 min, then raised to 150 mL/min for 10 min. The flow rate of drift gas and temperature in the drift tube were150 mL/min and 45 °C, respectively.

The retention index (RI) of volatile compounds was calculated using n-ketones C4-C9 as external references. Identification of volatile compounds was performed via the comparison of the RI and drift time based on the GC-IM library.

2.9. Statistical analysis

All data of this study were analyzed by using SPSS version 22.0 (SPSS Inc., Chicago, USA) with one-way analysis of variance. The differences among the four groups were analyzed by Tukey's test, and $P < 0.05$ indicated the significant difference. The results were denoted as means ± standard error (SE). The figures were plotted by GraphPad Prism 8.0 software. SIMCA 14.1 software (Umetrics, Umea, Sweden) was applied to analyze principal component analysis (PCA), orthogonal partial least square discriminant analysis (OPLS-DA), and variable importance in projection (VIP). Heatmap was generated in TBtools (v2.013). Correlation network model was performed by Cytoscape software (v.3.4.0).

3. Results and discussion

3.1. Chemical composition

There was no significant difference ($P > 0.05$) in the contents of moisture, crude protein, crude fat, and ash in breast muscle among the treatment groups (Supplementary Fig. 1). Therefore, it is suggested that dietary FPR had no significant effect on the chemical composition of breast muscle in yellow-feathered chickens.

3.2. Antioxidant capacity

As presented in Fig. 1. Compared with the control group, the contents of T-AOC and GSH-Px in breast muscle were significantly increased ($P < 0.05$) in the 4 % and 8 % FPR groups, and 8 % FPR diet also increased ($P < 0.05$) GSH-Px content than the 2 % FPR diet (Fig. 1A and B). While the SOD content in the 8 % FPR group was significantly higher ($P < 0.05$) than that in the control and 2 % FPR groups (Fig. 1C). Generally, excessive reactive oxygen species (ROS) can pose a serious threat to the integrality of cellular ultrastructure, which negatively affects health status and meat quality in poultry (Zaboli, Huang, Feng, & Ahn, 2019). The oxidative stress was alleviated primarily by increasing the activities of antioxidant enzymes, such as, T-AOC, GSH-Px, and SOD. T-AOC is an indicator of antioxidant capacity and can reflect the ability of scavenging free radicals. GSH-Px has ability to protect cells from damage of oxides. SOD can convert the superoxide anion to hydrogen peroxidase and oxygen, and which are detoxified to water by antioxidant enzymes. The current study showed that 4 % or 8 % FPR can improve antioxidant status of breast muscle in yellow-feathered chickens. Similarly, a recent report demonstrated that dietary fermented tomato pomace improved

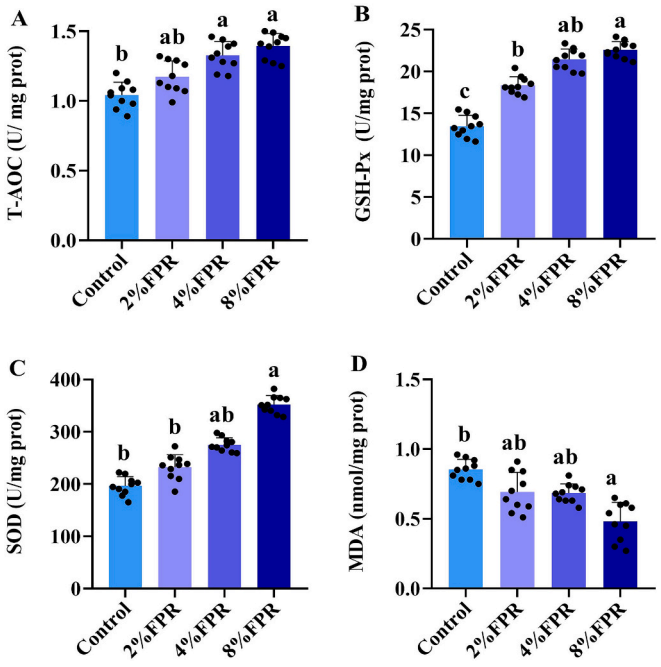

Fig. 1. Effects of fermented pineapple residue (FPR) on antioxidant capacity in breast muscle of yellow-feathered chickens. T-AOC: total antioxidant capacity; GSH-Px: glutathione peroxidase; SOD: superoxide dismutase; MDA: malondialdehyde. Control: chickens received a basal diet; 2 %, 4 %, and 8 % FPR: chickens received a basal diet with 2 %, 4 %, and 8 % FPR, respectively. Different letters indicate significant differences ($P < 0.05$). Data are presented as mean ± SE ($n = 10$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

levels of serum SOD and GSH-Px in broiler chickens (Gungor et al., 2024). Moreover, MDA is a major product of lipid peroxidation and its content reflects the degree of lipid peroxidation. In this study, the MDA content in the 8 % FPR group was significantly lower ($P < 0.05$) than in the control group (Fig. 1D). Consistent with this study, an earlier report found that pineapple peel was a potential antioxidant to retard lipid oxidation in beef meat (Lourenço, Fraqueza, Fernandes, Moldão-Martins, & Alves, 2020). It is known that pineapple residue contains high proportions of phenols and flavonoids (Mirabella et al., 2014). Indeed, the antioxidant capacity of fruit residues can be increased by fermentation, due to the improvement of the bioactive activities (Aziz & Karboune, 2018). These results suggest that FPR plays a significant positive role in antioxidant status of breast muscle in yellow-feathered chickens.

3.3. Meat quality traits

As shown in Fig. 2. Dietary supplementation with 4 % and 8 % FPR significantly increased ($P < 0.05$) the pH value of breast muscle compared to the control group (Fig. 2A). Muscle pH is a critical factor for meat quality and reflects the rate of muscle postmortem glycogenolysis, a decrease of pH leads to lactate accumulation and protein denaturation (Wang et al., 2020). The result of antioxidant capacity in this study has been confirmed that FPR can combat ROS production in chicken meat. Speculating that FPR protected cell membrane integration and prevented muscle rancidity by delaying lactate accumulation, finally, increasing the meat pH. The pH value is negatively correlated with the cooking loss. Supplementation with 8 % FPR significantly decreased ($P < 0.05$) the cooking loss of breast muscle than the control group (Fig. 2F). The decreased cooking loss represents an improvement in water-holding capacity (WHC) of breast meat, which is correlated to the nutrition, flavor, and juiciness of meat. However, dietary FPR supplementation did not affect ($P > 0.05$) meat color (L^*, a^*, and b^*), drip loss,

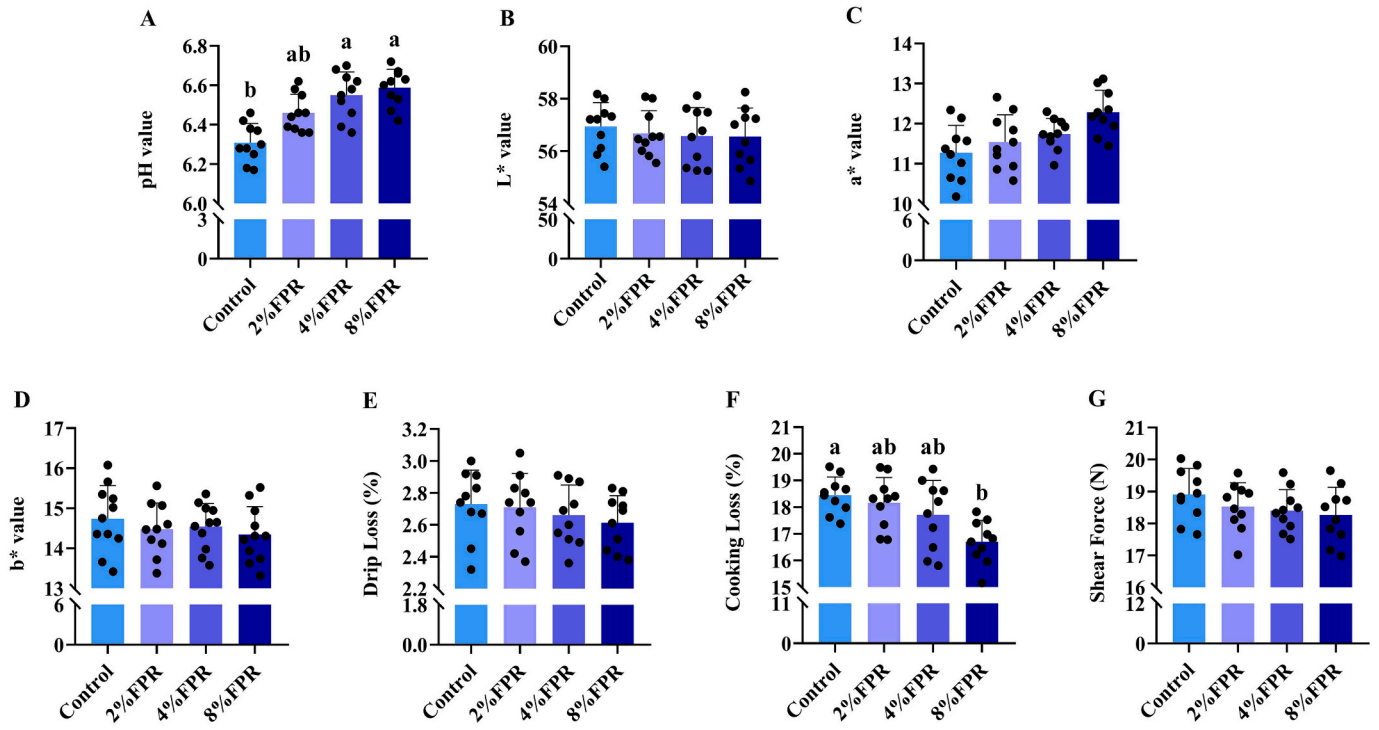

Fig. 2. Effects of fermented pineapple residue (FPR) on meat quality in breast muscle of yellow-feathered chickens. L^*: lightness; a^*: redness; b^*: yellowness. Control: chickens received a basal diet; 2 %, 4 %, and 8 % FPR: chickens received a basal diet with 2 %, 4 %, and 8 % FPR, respectively. Different letters indicate significant differences ($P < 0.05$). Data are presented as mean ± SE (n = 10). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and shear force of breast meat in yellow-feathered chickens (Fig. 2B, C, D, E, G). Dissimilar to the current finding, Deng et al. (2022) reported that FPR did not affect the meat quality in Simmental bull, this may be due to the different animal species. Surprisingly, another study revealed that the improvement of pH value and WHC was related to the increase in antioxidant capacity in broilers by dietary fermented *Ginkgo biloba* leaves (Cao, Zhang, Yu, Zhao, & Wang, 2012). According to these results, it could be speculated that the improvement in pH value and cooking loss could be potentially attributed to the antioxidant properties of FPR that are able to improve the oxidative stability in meat. It is therefore suggested that supplementation with FPR improved meat quality in yellow-feathered chickens.

3.4. Fatty acid profile

Fatty acid profile is an important parameter for evaluating the flavor

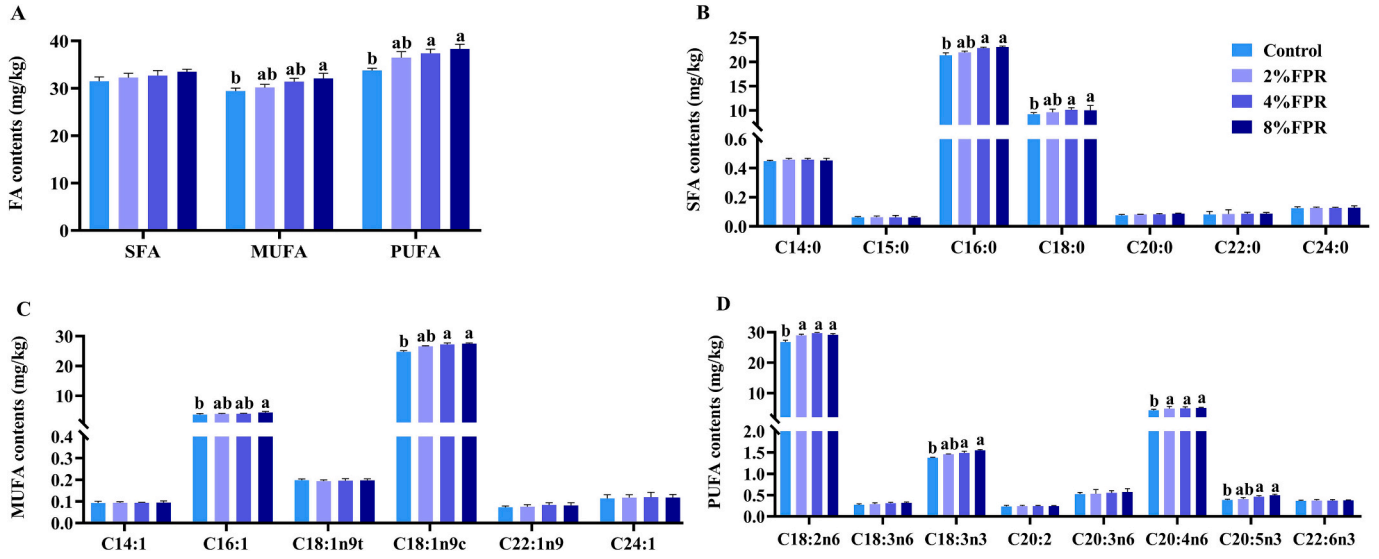

Fig. 3. Effects of fermented pineapple residue (FPR) on fatty acid composition in breast muscle of yellow-feathered chickens. FA: fatty acid; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid. Control: chickens received a basal diet; 2 %, 4 %, and 8 % FPR: chickens received a basal diet with 2 %, 4 %, and 8 % FPR, respectively. Different letters indicate significant differences ($P < 0.05$). Data are presented as mean ± SE (n = 10). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and nutritional value in meat. Dietary strategy is an effective way to alter fatty acid profile of meat. As shown in Fig. 3. Supplementation with 8 % FPR had a significant increase ($P < 0.05$) in the monounsaturated fatty acid (MUFA) content, while the polyunsaturated fatty acid (PUFA) contents in the 4 % and 8 % FPR groups were higher ($P < 0.05$) than in the control group. However, FPR groups had no effect on ($P > 0.05$) the saturated fatty acid (SFA) content (Fig. 3A) compared to the control group. UFA, especially PUFA, is the most susceptible to oxidization and cause the off-flavor of meat (Bartosz, 2013), and the antioxidant function of FPR has ability to neutralize lipid free radical. Similarly, Ahmed, Ko, and Yang (2017) reported that dietary supplementation with fermented pomegranate increased MUFA content and oxidative stability of in broiler meat. The current results indicate that the dietary FPR could increase the contents of MUFA and PUFA in breast msucle of yellow-feathered chickens.

According to Fig. 3B, compared to the control group, supplementation with 4 % and 8 % FPR increased ($P < 0.05$) the contents of palmitic acid (C16:0) and stearic acid (C18:0) of breast muscle. 8 % FPR significantly increased ($P < 0.05$) the palmitoleic acid (C16:1) content of breast muscle, and the oleic acid (C18:1n9c) content of breast muscle was greater ($P < 0.05$) in the 4 % and 8 % FPR groups than in the control group (Fig. 3C). Moreover, the contents of linoleic acid (C18:2n6c) and arachidonic acid (C20: 4n6) in breast muscle were greater ($P < 0.05$) by supplementation with 2 %, 4 %, and 8 % FPR, whereas the contents of α-linolenic acid (C18:3n3) and eicosapentaenoic acid (C20:5n3) in breast muscle were higher ($P < 0.05$) in the 4 % and 8 % FPR groups (Fig. 3D), compared to the control group. It is believed that palmitic acid and stearate acid were desaturated to palmitoleic and oleic acid by stearoyl-CoA desaturase. In addition, oleic acid can be converted to linoleic acid and α-linolenic acid, the substrate of arachidonic acid is linoleic acid, and α-linolenic acid can be metabolized to eicosapentaenoic acid (Abedi & Sahari, 2014). On the one hand, UFA is more susceptible to oxidation for generating meat aroma during thermal process (Han et al., 2023). On the other hand, UFA plays a main role in human health (Djuricic & Calder, 2021). Collectively, the changes in fatty acid profile are due to the improvement of antioxidant capacity in breast muscle. This suggests that FPR could improve meat quality and the nutritional value in yellow-feathered chickens.

3.5. Identification of volatile compound by GC-IMS

3.5.1. Topographic plots

The changes of volatile compounds in breast muscle of yellow-feathered chickens fed with FPR are presented by the topographic plots. As shown in three-dimensional (3D) topographic plot (Supplementary Fig. 2), the drift time of the separated ion, the retention time of substance, and the peak height of the ionic compounds were represented by the X-axis, Y-axis, and *Z*-axis, respectively. Each spot indicates one volatile compound. It can be observed that the distribution of ion peaks in the FPR groups were expanded than the control group. The differences of volatile compounds in breast muscle samples from yellow-feathered chickens could be distinguished, which was particularly reflected in volatile compound content after supplementation with FPR.

The 3D spectrum is not easy to distinguish the differences of volatile compounds between the control and FPR groups. In order to observe conveniently, the two-dimensional (2D) topographic plot is displayed in full-size as a top view to show the changes of volatile compounds among the four groups. Volatile compounds were located to the right side of the reactive ion peak (RIP) in breast muscle samples (Fig. 4A). The samples of the control group were used as a reference, and the samples of the FPR groups were subtracted from the reference. The color reflects the content of volatile compound, if a volatile compound is consistent with the reference, it appears white color, red color indicates the higher content of volatile compound, while blue color indicates the lower content of volatile compound compared to the reference. As presented in Fig. 4B, most signals of breast muscle samples appeared in the retention times of 100–700 s and drift times of 1.0–2.0 ms. This indicates that the contents of volatile compounds in breast muscle samples changed with the increase of FPR levels.

3.5.2. Fingerprints analysis

To further detect the changes of volatile compounds in breast muscle of yellow-feathered chickens fed with FPR. Fingerprinting profiles were generated automatically by Gallery plot. As shown in Fig. 4C, each row represents a sample, and each column represents a volatile compound in breast muscle samples of the four groups. The color reflects the content of volatile compound, the brighter signal indicates the higher content of volatile compounds (Wang, Chen, & Sun, 2020). Some compounds can generate two or three peak signals, which are referred to as monomer (M), dimer (D), or trimer (T). This due to the high proton affinity of the compounds and have ability to share ions for forming dimers or trimers when passing through the drift region (Liu et al., 2020). Forty-three volatile compounds were detected in breast muscle samples of yellow-feathered chickens fed with FPR via database of the instrumental software, including 13 aldehydes, 10 alcohols, 13 esters, 4 ketones, 1 acid, 1 furan, and 1 camphene (Supplementary Table 3). The content of most volatile compounds increased with the increase of FPR levels, with the exception of benzaldehyde and 2-Butanone, which were reduced with the increase of FPR (Table 1).

Aldehydes are the main products of lipid oxidation in meat and have lower odor thresholds (Sun et al., 2023). Most aldehydes content increased with the increase of FPR levels in breast muscle. The contents of hexanal, pentanal, nonanal, octanal, heptanal, 2-methylpentanal, and (E)-2-octenal were highest ($P < 0.05$) in breast muscle after supplementation with FPR. These compounds were decomposition products of fatty acids oxidation. For example, hexanal and heptanal, were mainly produced via the oxidation degradation of linoleic acid and arachidonic acid, while oleic acid can produce octanal and nonanal by oxidative degradation (Huang et al., 2022). Aldehydes are generally associated with fatty and grassy aromas (García-González, Aparicio, & Aparicio-Ruiz, 2013). Strangely, benzaldehyde content displayed a decrease trend with the increase of FPR. Benzaldehyde was product of amino acid phenylglycine by Strecker degradation and exhibited almonds flavor (Hidalgo & Zamora, 2019). This could be due to the feed ingredient was replaced by FPR in the diet, and amino acid contents reduce with the decrease of feed ingredient, leading to the decrease of benzaldehyde content.

Alcohols are generally produced via sugar metabolism lipid oxidation, decarboxylation and dehydrogenation of amino acids, and reduction of aldehydes with the pleasant fruity and floral aroma (García-González et al., 2013; Zhang, Hu, Wang, Kong, & Chen, 2021). Unsaturated alcohols are the important contributors of meat flavor with lower threshold value, while saturated alcohols exert little effect on meat flavor due to the higher odor threshold (Gu, Wang, Tao, & Wu, 2013). Such as, oct-1-en-3-ol and 2-hexanol had the highest contents ($P < 0.05$) in breast muscle of FPR groups and originated from the oxidation of PUFA (Fu, Cao, Yang, & Li, 2022). Most alcohol compounds in breast muscle increased with the increase of FPR levels in yellow-feathered chickens.

Esters are obtained by the oxidative esterification of alcohols and fatty acids (García-González et al., 2013). Ester compounds have lower odor thresholds value and fruity aroma, which are the main volatile components of meat (Flores, Corral, Cano-García, Salvador, & Belloch, 2015). Most ester compounds of breast muscle increased ($P < 0.05$) with the increase of FPR levels in yellow-feathered chickens. Thus, the increased ester contents can be explained by the increases of fatty acid contents and alcohol compounds, contributing to the ester compounds development in yellow-feathered chickens.

Ketones are products of lipid oxidation, amino acid degradation, and Maillard reaction and have the higher threshold value, which is considered to be as the precursor of fatty flavor and play a coordinating action in the formation of meat aroma (Chung, Yung, Ma, & Kim, 2002).

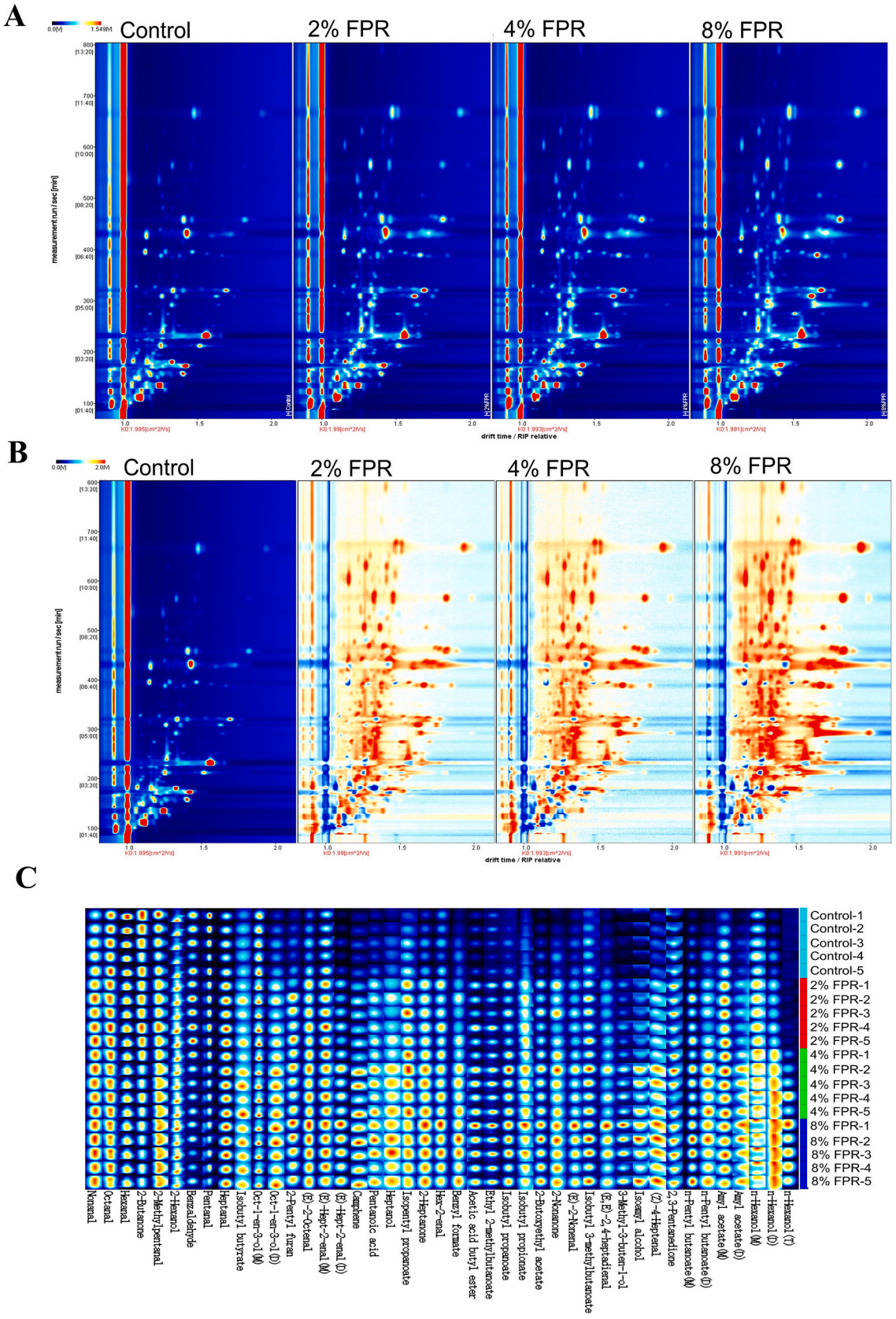

Fig. 4. Analysis of GC-IMS in yellow-feathered chickens fed with fermented pineapple residue (FPR). (A) Two-dimensional topographic plots; (B) Two-dimensional difference comparison plots; (C) Fingerprint plot of volatile compounds. Control: chickens received a basal diet; 2 %, 4 %, and 8 % FPR: chickens received a basal diet with 2 %, 4 %, and 8 % FPR, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Volatile compounds in breast muscle of yellow-feathered chickens fed with FPR.

Compounds	Peak intensity			
	Control	2 % FPR	4 % FPR	8 % FPR
Aldehydes				
Pentanal	5473.49 ± 43.54^{c}	6075.28 ± 71.58^{bc}	6470.01 ± 123.59^{ab}	7051.25 ± 247.94^{a}
Hexanal	9094.22 ± 246.63^{c}	9646.13 ± 126.29^{bc}	10,249.45 ± 35.60^{ab}	10,606.92 ± 226.91^{a}
Heptanal	1797.27 ± 120.01^{c}	2840.12 ± 115.62^{b}	3315.16 ± 76.45^{ab}	3439.37 ± 132.48^{a}
Octanal	2335.36 ± 98.51^{b}	2750.57 ± 78.44^{a}	2734.8 ± 34.06^{a}	2771.61 ± 10.34^{a}
Nonanal	2215.8 ± 121.75^{b}	3218.78 ± 176.50^{a}	3544.51 ± 80.34^{a}	3519.04 ± 99.62^{a}
Hex-2-enal	269.47 ± 19.27^{c}	418.37 ± 18.62^{b}	485.54 ± 18.25^{ab}	580.08 ± 32.09^{a}
(*E*)-2-Nonenal	185.09 ± 24.30^{c}	373.08 ± 16.57^{b}	498.58 ± 39.98^{ab}	549.03 ± 55.20^{a}
(E)-2-Octenal	1049.89 ± 61.63^{b}	2050.73 ± 77.96^{a}	2439.75 ± 145.59^{a}	2650.99 ± 155.44^{a}
2-Methylpentanal	1529.11 ± 46.71^{c}	1858.72 ± 65.23^{b}	2472.73 ± 100.14^{a}	2589.61 ± 23.60^{a}
(E)-Hept-2-enal (M)	843.97 ± 31.04^{c}	1247.06 ± 29.34^{b}	1367.37 ± 38.24^{ab}	1468.71 ± 27.14^{a}
(E)-Hept-2-enal (D)	308.43 ± 27.16^{c}	1080.36 ± 43.31^{b}	1802.14 ± 171.88^{a}	1976.75 ± 227.97^{a}
Benzaldehyde	671.03 ± 8.83^{a}	689.08 ± 20.44^{a}	523.6 ± 11.62^{b}	472.81 ± 47.93^{b}
(E, E)-2,4-heptadienal	59.7 ± 5.97^{c}	138.79 ± 7.08^{bc}	195.75 ± 24.62^{ab}	230.99 ± 29.62^{a}
Alcohols				
n-Hexanol(M)	927.23 ± 14.02^{b}	1489.51 ± 11.98^{a}	1686.35 ± 42.57^{a}	1518.56 ± 63.73^{a}
n-Hexanol(D)	174.15 ± 11.83^{c}	521.32 ± 24.59^{b}	936.84 ± 27.33^{a}	1004.14 ± 23.58^{a}
n-Hexanol(T)	24.37 ± 2.03^{c}	59.09 ± 4.62^{c}	244.41 ± 36.92^{b}	434.36 ± 27.10^{a}
Heptanol	142.41 ± 15.65^{c}	297.9 ± 25.09^{b}	402.64 ± 12.20^{ab}	446.7 ± 33.19^{a}
2-Hexanol	3422.96 ± 38.94^{c}	4205.68 ± 72.27^{b}	4179.87 ± 89.05^{b}	4680.12 ± 119.62^{a}
Oct-1-en-3-ol (M)	2577.17 ± 70.33^{b}	2818.52 ± 85.81^{ab}	2874.47 ± 55.32^{a}	2880.98 ± 38.29^{a}
Oct-1-en-3-ol (D)	519.07 ± 34.34^{c}	1044.21 ± 53.22^{b}	1430.21 ± 16.45^{a}	1333.87 ± 44.34^{a}
Isoamyl alcohol	266.05 ± 10.20^{c}	497.88 ± 13.33^{b}	925.64 ± 43.41^{a}	835.04 ± 33.20^{a}
(Z)-4-Heptenal	359.17 ± 16.39^{c}	778.97 ± 20.82^{b}	1116.04 ± 23.97^{a}	1049.89 ± 51.82^{a}
3-Methyl-3-buten-1-ol	41.13 ± 2.04^{b}	65.67 ± 5.47^{b}	132.73 ± 11.70^{a}	149.73 ± 17.78^{a}
Esters				
Benzyl formate	154.63 ± 17.35^{c}	393.76 ± 49.89^{b}	611.72 ± 33.04^{a}	604.33 ± 39.47^{a}
Acetic acid butyl ester	114.63 ± 7.01^{c}	281.66 ± 17.87^{b}	455.96 ± 12.97^{a}	481.88 ± 36.03^{a}
Amyl acetate (M)	129.27 ± 6.33^{b}	291.53 ± 8.32^{a}	330.41 ± 9.76^{a}	334.64 ± 11.70^{a}
Amyl acetate (D)	63.62 ± 5.47^{c}	161.2 ± 4.83^{b}	249.85 ± 14.44^{a}	279.98 ± 18.77^{a}
n-Pentyl butanoate (M)	796.87 ± 188.14^{b}	2273.48 ± 371.09^{a}	3476.16 ± 341.14^{a}	3184.47 ± 324.84^{a}
n-Pentyl butanoate (D)	90.76 ± 5.12^{d}	268.06 ± 6.13^{c}	401.98 ± 12.44^{a}	332.03 ± 11.80^{b}
Isobutyl propanoate	47.25 ± 4.60^{c}	91.58 ± 5.10^{bc}	124.47 ± 11.38^{ab}	145.97 ± 10.87^{a}
Isopentyl propanoate	111.86 ± 8.97^{b}	258.07 ± 16.82^{a}	292.59 ± 12.04^{a}	265.74 ± 11.39^{a}
Isobutyl propionate	251.5 ± 16.22^{b}	527.24 ± 20.23^{a}	558.39 ± 28.44^{a}	563.81 ± 35.45^{a}
Isobutyl butyrate	182.07 ± 7.53	231.41 ± 11.01	217.08 ± 10.17	226.35 ± 8.27
Ethyl 2-methylbutanoate	61.57 ± 4.02^{c}	133.83 ± 10.01^{b}	198.81 ± 7.07^{a}	215.66 ± 16.11^{a}
2-Butoxyethyl acetate	188.59 ± 25.11^{c}	650.96 ± 53.78^{b}	833.96 ± 58.42^{ab}	882.77 ± 43.89^{a}
Isobutyl 3-methylbutanoate	160.68 ± 15.56^{b}	330.88 ± 28.09^{a}	445.52 ± 23.40^{a}	388.87 ± 29.36^{a}
Ketones				
2-Butanone	6861.76 ± 381.90^{a}	6008.04 ± 173.49^{a}	4593.61 ± 60.10^{b}	4798.16 ± 256.36^{b}
2-Heptanone	712.36 ± 12.98^{c}	1964.2 ± 19.21^{b}	2676.56 ± 57.74^{a}	2701.7 ± 73.94^{a}
2-Nonanone	85.24 ± 5.01^{b}	313.23 ± 34.15^{a}	356.17 ± 26.45^{a}	364.56 ± 18.21^{a}
2,3-Pentanedione	924.09 ± 29.24^{b}	1144.97 ± 27.29^{ab}	1291.85 ± 42.55^{a}	1333.44 ± 55.62^{a}
Others				
2-Pentyl furan	332.21 ± 16.45^{b}	652.5 ± 27.08^{ab}	780.24 ± 44.57^{a}	787.35 ± 69.13^{a}
Pentanoic acid	86.18 ± 7.27^{c}	221.64 ± 6.92^{b}	316.95 ± 10.99^{a}	276.12 ± 24.22^{ab}
Camphene	133.45 ± 11.50^{c}	342.4 ± 20.19^{b}	561.28 ± 18.14^{a}	500.96 ± 15.66^{a}

Control: chickens received a basal diet; 2 %, 4 %, and 8 % FPR: chickens received a basal diet with 2 %, 4 %, and 8 % fermented pineapple residue, respectively. Different letters indicate significant differences ($P < 0.05$).

In this study, 2-heptanone, 2-nonanone, and 2,3-pentanedione were increased ($P < 0.05$) with the increase of FPR levels. 2-butanone gradually decreased ($P < 0.05$) when FPR replaced the feed ingredient in the diet. Speculating that 2-butanone may be derived from feed ingredient.

Furthermore, 2-pentylfuran and pentanoic acid were increased ($P < 0.05$) in breast muscle by the addition of FPR. 2-pentylfuran is oxidative product of linoleic acid, pentanoic acid is produced by low-grade fatty acids and has higher odor threshold, which contributes to the minor effect on meat flavor (Jiang et al., 2022). In addition, camphene was higher ($P < 0.05$) in breast muscle after supplementation with FPR in this study. Previous study demonstrated that terpene volatile compounds can be transferred to meat via diet (Coppa et al., 2011). As expected, the terpenes were found in pineapple (Sharma, Dhami, & Pandey, 2014). Speculating that terpene comes directly from FPR.

3.6. Multivariate analysis of volatile compound in breast muscle

3.6.1. PCA and OPLS-DA analysis

PCA and OPLS-DA analysis were performed to better investigate the differences of volatile compounds in breast muscle samples of yellow-feathered chickens fed with FPR. In PCA model, the contribution of PC1 and PC2 reached 89 % (Fig. 5A), indicating that most of the information of the original variables can be retained by dimension reduction process. PC1 separated the control and 4 % and 8 % FPR groups while PC2 separated the control and 2 % FPR group. This indicates that volatile compound contents of the control group were completely different from those of the 2 %, 4 %, and 8 % FPR groups, respectively. However, the samples were not clearly distinguished between the 4 % and 8 % FPR groups, indicating that volatile compounds content were similar between the 4 % and 8 % FPR groups.

OPLS-DA analysis was to further identify the characteristic distribution of volatile compounds in the breast muscle of yellow-feathered chickens fed with FPR (Fig. 5B). OPLS-DA model showed that the R^2Y and Q^2 were 0.838 and 0.633, respectively, indicating that this model has a strong cumulative interpretation and predictive abilities. OPLS-DA model presented a better classification in volatile compounds of yellow-feathered chickens fed with FPR. To prove whether OPLS-DA was

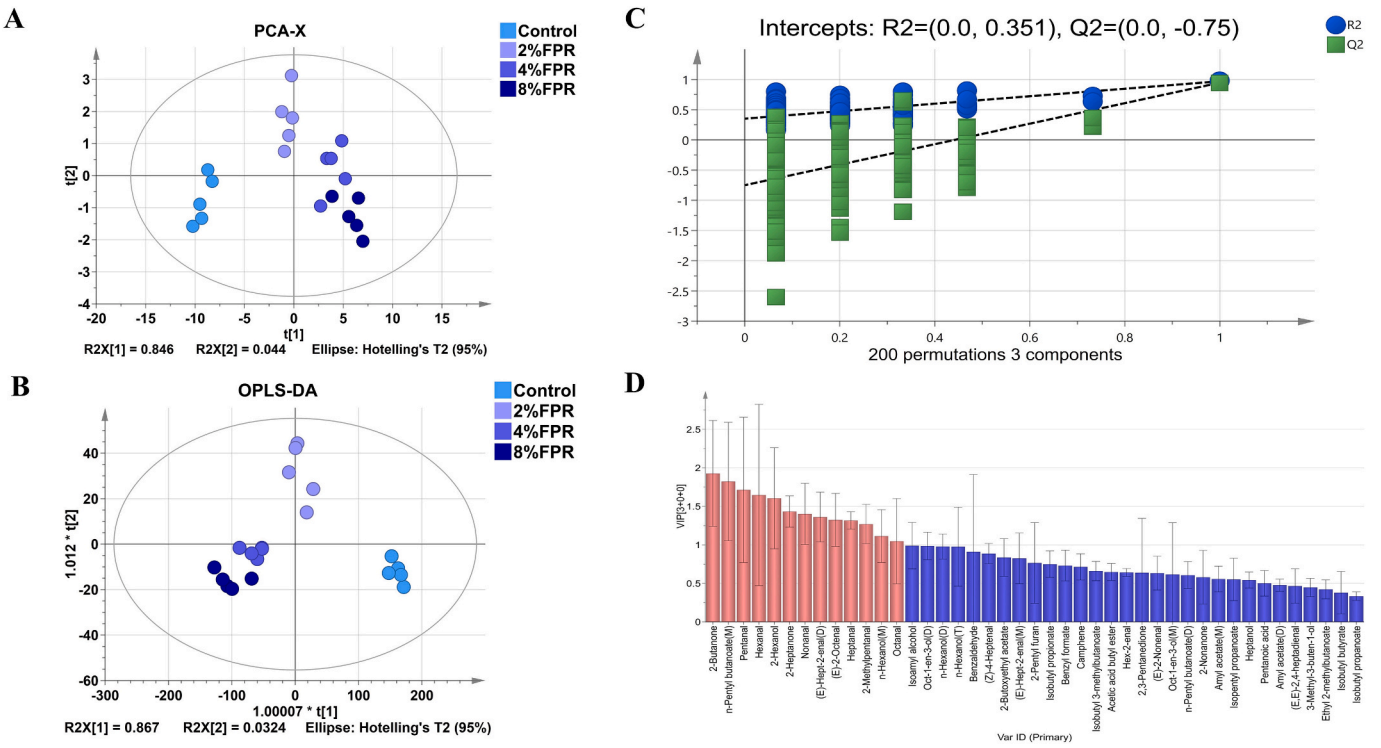

Fig. 5. Multivariate analysis of volatile compounds in breast muscle of yellow-feathered chickens fed with fermented pineapple residue (FPR). (A) Principal component analysis (PCA) score plot. (B) Orthogonal partial least squares discriminant (OPLS-DA) score plot. (C) Permutation test plot of OPLS-DA model. (D) Variable importance in projection (VIP) in OPLS-DA model. Control: chickens received a basal diet; 2 %, 4 %, and 8 % FPR: chickens received a basal diet with 2 %, 4 %, and 8 % FPR, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

overfitting and effectiveness, the permutation test (200 times) was performed (Fig. 5C), showing that the intercept values of R^2 and Q^2 were 0.351 and − 0.75, indicating that OPLS-DA is not over-fitting and reliable. Thus, OPLS-DA is suitable approach to assess the differences of volatile compounds in yellow-feathered chickens fed with FPR. Speculating that FPR supplementation can affect volatile compounds in breast muscle of yellow-feathered chickens.

3.6.2. *VIP analysis*

The contribution of each compound was analyzed by VIP in OPLS-DA model, and the key volatile compounds were screened as potential markers in breast muscle samples of yellow-feathered chickens fed with FPR. The higher VIP value represents the larger contribution. The volatile compounds with VIP > 1.0 were selected as the great contributors in breast muscle samples of yellow-feathered chickens fed with FPR. As seen in Fig. 5D, 13 volatile compounds were screened out, including 2-butanone, n-hexanol (M), nonanal, octanal, 2-hexanol, hexanal, pentanal, (E)-hept-2-enal (D), 2-methylpentanal, n-pentyl butanoate (M), (E)-2-octenal, 2-heptanone, and heptanal (Fig. 6). Most volatile compounds were aldehydes, implying that aldehyde compounds make a great contribution for meat flavor formation. Suggesting that this could be served as potential markers to distinguish volatile compounds in breast muscle of yellow-feathered chicken fed with FPR.

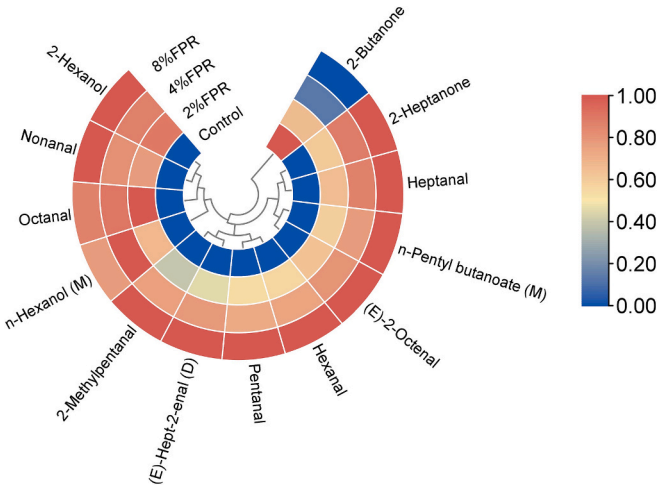

Fig. 6. Heat map clustering of key volatile compounds (VIP > 1) in breast muscle of yellow-feathered chickens fed with fermented pineapple residue (FPR). Control: chickens received a basal diet; 2 %, 4 %, and 8 % FPR: chickens received a basal diet with 2 %, 4 %, and 8 % FPR, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.7. *Correlation between fatty acids and key volatile compounds*

Volatile compounds are produced by the oxidative degradation of fatty acids. Herein, UFA are the main precursors of meat flavor (Shahidi & Hossain, 2022). Therefore, Pearson correlation coefficients (|r| > 0.7) between fatty acids and 13 volatile compounds (VIP > 1) was plotted (Fig. 7). The results revealed that PUFA and C18:1n9c showed a highly significant positive correlation with 12 volatile compounds (nonanal, n-Pentyl butanoate (M), pentanal, hexanal, 2-hexanol, 2-heptanone, (E)-Hept-2-enal (D), (E)-2-Octenal, heptanal, 2-methylpentanal, n-Hexanol (M) and octanal), respectively. C18:2n6 was highly significantly positively associated with (*E*)-hept-2-enal (D) and (E)-2-octenal, respectively. While a negative correlation was found between C18:1n9c and 2-butanone (Supplementary Table 4). Oleic acid (C18:1) and linoleic acid (C18:2) are the UFAs, which contribute to the formation of aldehydes and alcohol by oxidative process (Shahidi, 1994). This is supported by the report of Huang et al. (2022), indicating that linoleic acid produced

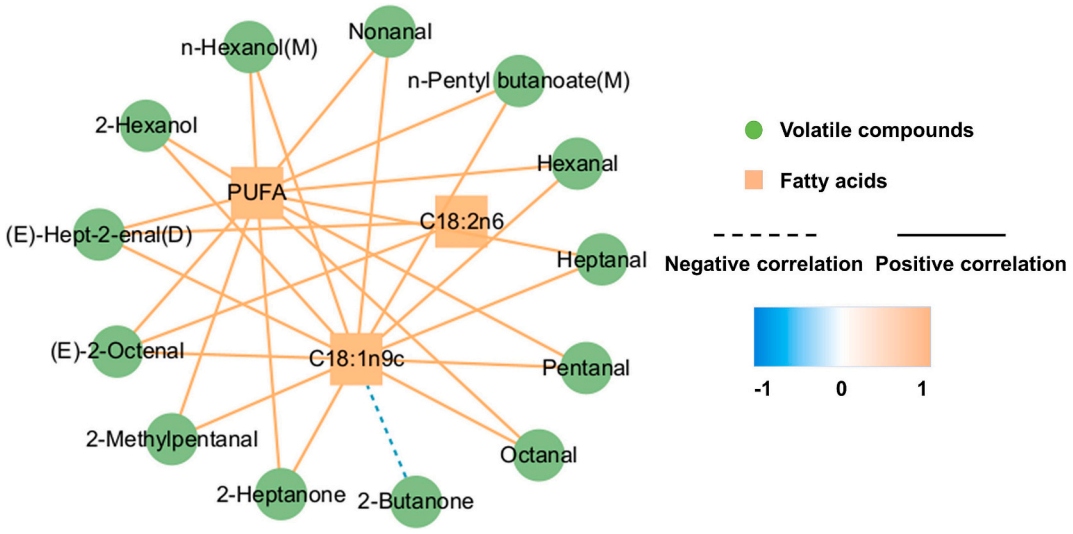

Fig. 7. Correlation network diagram between fatty acids and volatile compounds (VIP > 1). Green circles represent volatile compounds, orange squares represent dominant fatty acids ($|r| > 0.7$ and $P < 0.05$). Orange solid line and blue dotted line represent positive and negative correlation, respectively. PUFA: polyunsaturated fatty acid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

hexanal and heptanal by oxidative degradation, while octanal and nonanal were produced by degradation of oleic acid. Fu et al. (2022) demonstrated that the saturated aldehydes, such as hexanal and nonanal were derived from the oxidation of linoleic acid. The current result was consistent with previous finding, reporting that C18:1 n9c and C18:2 n6 were the important precursors in flavor improvement (Han et al., 2023). Especially, PUFA were in volved in formation of aldehydes. It is suggested that FPR can improve the contents of C18:1n9c, C18:2n6, and PUFA, contributing to the enhancement of meat flavor in yellow-feathered chickens.

4. Conclusion

In conclusion, dietary FPR supplementation improved meat quality and altered fatty acid profile by the increase of antioxidant capacity. GC-IMS detected 43 volatile compounds and 12 volatile compounds (n-hexanol (M), nonanal, octanal, 2-hexanol, hexanal, pentanal, (E)-hept-2-enal (D), 2-methylpentanal, n-pentyl butanoate (M), (E)-2-octenal, 2-heptanone, and heptanal) could serve as potential aroma markers to distinguish meat flavor in breast muscle of chickens fed with FPR. Correlation analysis between fatty acids and key volatile compounds revealed that C18:1n9c, C18:2n6, and PUFA are the important contributors for meat flavor formation. This study suggests that FPR could be used as feed additive, contributing to the improvement of meat quality in yellow-feathered chickens.

CRediT authorship contribution statement

Panpan Lu: Writing – original draft, Data curation, Formal analysis. **Ruiting Guo:** Investigation, Methodology. **Chunlian Zou:** Investigation, Methodology. **Hang Chen:** Investigation, Software. **Dan Chen:** Supervision. **Lu Yang:** Supervision. **Huize Tan:** Supervision. **Siqiao Wu:** Investigation. **Yaxue Lv:** Investigation. **Zhengzhong Xiao:** Supervision. **Chunqi Gao:** Writing – review & editing, Supervision, Funding acquisition, Project administration.

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.2024.101874.

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