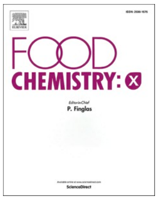
Food Chemistry: X 22 (2024) 101330

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Dietary effect of *Dendrobium officinale* leaves on chicken meat quality, fatty acid composition, and volatile compounds profile

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ARTICLE INFO

Keywords:
Dendrobium officinale leaves
Broiler
Meat quality
Fatty acids
Volatile compounds

ABSTRACT

Dendrobium officinale leaves (DOL) contain many active ingredients with various pharmacological effects, but are still ineffectively utilized. To investigate the feasibility of developing DOL as a feed additive, it is necessary to determine whether dietary supplementing DOL had any effect on meat quality and flavor. Our results showed that supplementation with DOL decreased the shear force while increased the pH and fat content in breast meat. Meat from DOL-fed chickens had higher levels of n-3 polyunsaturated fatty acids (PUFAs) and n-6 PUFAs, but lower n-6/n-3 ratios. Moreover, volatile compounds profile indicated that contents of aldehydes, including hexanal, pentanal, and heptanal, etc.), which were identified as the key volatile compounds in chicken meat, exhibited noteworthy rise in DOL intake groups. Octanal, 1-octen-3-ol, and 2-pentylfuran also contributed greatly to the meat overall aroma. These data provide a foundation for the comprehensive utilization of DOL as a feed additive with antibiotic substitution potential.

1. Introduction

Dendrobium officinale Kimura et Migo (*D. officinale*), belonging to the family Orchidaceae, is a traditional precious medical herb that has been widely used for centuries in China and the southeast Asian countries. The stems are officially recognized as the medicinal parts of *D. officinale* in the *Chinese Pharmacopoeia*. Other parts, like leaves, contain active components similar to those found in the stems (Youyuan et al., 2017), but remain underutilized as they are directly discarded during the harvesting of *D. officinale* stems.

Recently, several researches revealed that the leaves of *D. officinale* (DOL) also have many pharmacological activities, including immunomodulatory (Xie et al., 2022), alleviate hyperglycemia (Fang et al., 2022), and antioxidant (Zhang et al., 2017). Thus, there has been a significant increase in awareness and interest in DOL. On the other hand, with the development and improvement of artificial cultivation technology in recent decades, as well as the continuous excavation of the medicinal values of *D. officinale*, the cultivation scale of *D. officinale* has been expanding, and the annual output continues to rise, resulting in more and more wastage of the leaves. Therefore, it is very necessary to effectively explore and utilize the leaves.

Due to its high content of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), poultry meat is considered one of the greatest sources of dietary animal protein in the human diet worldwide, especially in developed countries (Zarate, El Jaber-Vazdekis, Tejera, Perez, & Rodriguez, 2017). With the growing population and rising household incomes, the demand for poultry meat, which is relatively more affordable than other meats in the market, has increased and forced the poultry industry to grow rapidly. Currently, promoting growth performance and improving product quality are the two major directions for rapidly producing meat product while satisfying consumer demand (Salter, 2017). In the past, the former was usually achieved through the use of growth-promoting antibiotics; with the prolonged utilization of antibiotics, however, resulting in the occurrence of drug-resistant bacteria, which seriously jeopardizes the environment and human health, and antibiotics have now been banned from use in livestock production (Aminov, 2010). To address this challenge, research has shifted towards the investigation of replacing antibiotics with natural bioactive

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https://doi.org/10.1016/j.fochx.2024.101330
Received 2 February 2024; Received in revised form 15 March 2024; Accepted 21 March 2024

compounds contained in plants. Particularly, some herbal extracts and by-products rich in compounds, mainly polysaccharides, phenols, and flavonoids, such as *Astragalus* (Qiao et al., 2022) and *Acanthopanax senticosus* (Long et al., 2021), have attracted much interest and have been utilized to ameliorate the health and growth performance of animals. With this background, we wondered if DOL could also be used as an additive to substitute antibiotics in animal feeds. Our previous study confirmed that dietary supplementation of the DOL to broiler diets promoted the growth performance, antioxidant activities, and the intestinal health of broilers, suggesting that the DOL may be a new potential resource for feed additives, and providing a new view for the utilization of DOL (Zhao et al., 2023).

On the other hand, product quality has always been the first concern of consumers. Meat quality-related parameters, including meat color, nutrient content, fatty acid composition, and volatile flavor, etc., are all factors that influence consumers' purchase willingness (Zotte & Szendrő, 2011). And improving these parameters is a goal pursued by researchers and the livestock industry. The feed supplementation strategy, a crucial connection between animal production, food technology, and human nutrition, has been identified as one of the primary factors influencing the parameters of meat quality (Mendonca et al., 2020). Many studies have found that natural agents also have a role in changing meat quality, for instance, adding sea buckthorn leaves (Saracila et al., 2022) to the diet in combination with Cr could significantly improve the fatty acid profile and the oxidative stability of chicken breast meat. We hypothesized that the utilization of DOL as a feed additive may have an impact on broiler meat quality.

Therefore, this investigation sought to figure out the changes in meat quality, chemical composition, fatty acid profile, as well as volatile flavor characteristics of the breast muscle following the DOL addition to broiler diets.

2. Materials and methods

2.1. Animal management, experimental design and meat samples collection

This experiment was performed at a poultry farm located in Xianju county, Taizhou City, Zhejiang Province, China. A total of 144 one-day-old male Xianju chickens were selected and randomly divided into 3 groups, each containing 6 replicates with 8 chickens per replicate. The control group (CON) was composed of broiler receiving a basal diet, which had ingredients and nutrient components listed in Table 1. Birds in two treatment groups (DL1 and DL2) were fed the test diets, i.e., basal diet supplemented with 1% and 5% of DOL throughout the testing period, respectively. The chicks were housed in 100 × 45 × 70 cm tiered three-tier cages, each equipped with a feeder and waterer, and reared under controlled environmental conditions. Feed and water were available ad libitum. The temperature was ensured to be 33 °C for the first 3 days, after which it was lowered by 1 °C every other day, gradually to 24 °C, and then maintained until the end of the experiment.

On day 70, after an overnight fast for 12 h, 12 chickens from each group were randomly selected, stunned with a stun bath (voltage: 30–50 V), and exsanguinated by severing the jugular vein and carotid artery on one side of the neck. After dissection, evisceration, and determination of carcass traits, breast meat samples (pectoralis major) were collected and determined the physical parameters of meat, including pH, shear force and meat color. The remaining portions of the breast meat were frozen in sealed polythene bags and transported to the laboratory on ice for assessment of proximate composition (dry matter, DM; crude protein, CP; crude fat, CF; ash), fatty acids profile, and volatile components.

2.2. Meat quality assessment

The pH values of the same section of breast muscle samples were determined by inserting the electrode of a digital pH meter (pH-STAR, MATTHAUS, Germany) into the meat samples at approximately 1.0–1.5 cm within 15 min after slaughtering, and each meat sample was measured in triplicate. Prior to use, the instrument was calibrated with a standard phosphate buffer (pH 4.00 and 7.00) and the electrode was carefully rinsed with distilled water at the end of each measurement before the next measurement.

Parameters for evaluating the meat color, including lightness (L^*), redness (a^*), and yellowness (b^*) values were determined using a portable colorimeter (Chroma Meter CR-410; Konica Minolta, Japan) with pulsed xenon arc lamp, a D65 illuminant, a standard observer at 10°, and CIE L*a*b* color scale. All assessments were carried out at three times.

Strips with dimensions of 1 × 1 × 3 cm were cut from the meat samples along the direction of the muscle fibers, and shear force was measured using a Warner-Bratzkr Meat Shear (G-R151, G-R Co., USA), which has a 50 kg load transducer and a cutter head with a constant speed of 225 mm/min. The maximum force values were recorded as the shear force when the breast meat samples were cut.

Approximately 20 g of tissue was taken from the same spot of breast muscle samples, weighted (W1), placed into individual Ziplock bag, and heated in a water bath at 75 °C for 20 min. The samples were then cooled under running water, the residual moisture on surface was absorbed with filter paper, then the samples were weighted again (W2). Cooking loss was expressed as the percentage: (W1-W2)/W1 × 100%.

Table 1
Ingredient composition and nutrient level of basal diets (%, as-fed basis).

Items	Day 1 to 42	Day 43 to 70
Ingredients (%)		
Corn	53.7	35.6
Soybean meal	23	8.2
Extruded soybean	6	2
Rice bran	6.5	6
Soybean oil	0.8	1.4
Corn gluten meal	3	4
Limestone	1.33	1.3
Premix[a]	4	3.2
Fermented soybean meal	1.67	
Wheat grain		18
Rice meal		10
DDGS (corn)[b]		10
Wheat red dog		0.3
Total	100	100
Calculated nutrient components		
Metabolizable energy(Kcal/kg)	2950	2997
Crude protein	21.1	16.7
Crude fat	4.8	5.5
Lysine	1.22	0.95
Methionine	0.54	0.40
Methionine and Cysteine	0.88	0.72
Threonine	0.85	0.67
Tryptophan	0.22	0.19
Calcium	0.87	0.70
Total phosphorus	0.63	0.58
Analyzed nutrient components		
Crude protein	21.12	16.34
Crude fat	4.89	5.58
Crude ash	5.04	5.53
Dry matter	89.75	90.24

[a] Premix provided per kilogram of diet: vitamin A, 1000 IU; vitamin D_3, 250 IU; vitamin E (DL-α-tocopheryl acetate), 15 mg; vitamin B_1, 3.6 mg; vitamin B_2, 2.8 mg; vitamin B_6, 4.1 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 7.5 mg; Fe (as $FeSO_4 \cdot 7H_2O$), 75 mg; Zn (as $ZnSO_4$), 51.75 mg; Mn (as $MnSO_4$), 55.65 mg; I (as $Ca(IO_3)_2$), 0.1 mg; Se (as $NaSeO_3 \cdot 5H_2O$), 0.05 mg.

[b] DDGS: Distillers dried grains with solubles.

2.3. Determination of chemical composition

The determination of primary chemical composition, including moisture, ash, crude protein, and crude fat in breast muscle samples were carried out following the methods recommended by the National Food Safety Standards of China (GB 5009.3-2016, GB 5009.4-2016, GB 5009.5-2016, GB 5009.6-2016), respectively.

2.4. Fatty acid composition analysis

The fatty acid composition of breast muscle samples was determined by gas chromatography referring to the approach described by Valentini et al. (Valentini et al., 2020) with slight adjustments. In brief, approximately 20 g of each meat sample was weighted, dried, and ground, and the total lipids were then extracted using a mixture of chloroform and methanol (2:1, *v*/*v*). An amount of 60 mg of extracted fat was fully redissolved with 4 mL isooctane, and the esterification of fatty acids was performed by the transesterification method, that was, 200 μL potassium hydroxide-methanol solution was added, violently shaken for 20 s, and left until clarified. Subsequently, 1 g of sodium bisulfate was added to neutralize the remaining potassium hydroxide. After salt precipitation, the supernatant was collected, filtered through a 0.22-μm micron membrane, and then detected on a gas chromatograph (Model 7890 A, Agilent Technologies, Palo Alto, CA, USA), which was equipped with a flame ionization detector (FID) and automated injection system. The SP-2380 column (Anpel Laboratory Technologies Inc., Shanghai, China) (100 m × 0.25 mm × 0.20 μm) was used to separate the analyses. The gas chromatograph conditions were as follows: the injection volume was 1.0 μL, the splitting ratio was 10:1; the carrier gas was high-purity helium at a flow rate of 1.0 mL/min; inlet and detector temperatures were 270 °C and 280 °C, respectively. The followed procedural ramp-up was used: the initial temperature was set at 100 °C and held for 13 min, then increased to 180 °C at 10 °C/min and held for 6 min, to 200 °C at 1 °C/min and held for 20 min, and to 230 °C at 4 °C/min and held for 10.5 min. Fatty acid methyl esters were identified by comparison with retention times of the authentic external standards, and expressed as g/kg of fatty acid methyl esters.

2.5. GC–MS analysis of volatile flavor compounds

2.5.1. Headspace solid-phase microextraction-gas chromatograph-mass spectrometry analysis

Volatile flavor compounds were determined using headspace solid-phase microextraction (HS-SPME) method established by Jin (Jin et al., 2021) with slight modification. The breast muscle sample (~ 5 g) was placed in a 20 mL glass headspace vial and immediately capped, then extracted on a solid-phase microextraction (SPME) fully automated loading system (PAL RTC 120, Agilent Technologies, Palo Alto, USA) at a heating temperature of 60 °C, preheated for 5 min and incubated for 30 min to extract the aroma substances. After headspace extraction, the SPME Arrow was inserted into the GC injection port and desorbed for 4 min.

Subsequently, samples were analyzed in an Agilent 7890B gas chromatograph system coupled with an Agilent 5977B mass spectrometer (GC–MS) utilizing an Agilent DB-Wax capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Palo Alto, USA) on splitless mode. The carrier gas was helium with high purity (99.99%) at a flow rate of 1 mL/min. Following was the procedure of the oven temperature ramp: the column temperature was initially maintained at 4 °C for 4 min, then gradually increased to 245 °C at a rate of 5 °C/min, holding for an additional 5 min. The ion source and quad temperatures were set at 230 °C and 150 °C, respectively, while the front injection and transfer line temperatures were both set at 250 °C. The energy was −70 eV in electron impact mode. With a solvent delay time of 2.13 min, the mass spectrometry data were obtained in scan mode, covering the *m*/*z* range of 20–400.

Utilizing Chroma TOF 4.3× software of LECO Corporation, based on the National Institute of Standards and Technology (NIST) database, each detected peak was qualitatively identified through a series of processes including raw peak exacting, data baseline filtering and baseline calibration, peak alignment, deconvolution analysis, peak identification, integration and spectrum matching of the peak area. The Peak Area Normalization method was applied to determine the relative content of each volatile compound, which was finally expressed as a proportion of each peak area to the total peak area.

2.5.2. Identification of flavor compounds

The relative odor activity value (ROAV) is commonly used to assess the contribution of individual volatile compound to the overall aroma, thereby determining the key volatile flavor compounds in broiler breast meat. Usually, the compound with the most pronounced influence on the overall flavor is defined as having a $\mathrm{ROAV}_{\max} = 100$, and the following formula is used to calculate the ROAV values of other compounds: $\mathrm{ROAV}_i = (C_i/C_{\max}) \times (T_{\max}/T_i) \times 100$, where C_i and T_i represent the relative content and odor threshold of the target flavor compound, respectively; $C_{\max}$ and $T_{\max}$ denote the relative content and odor threshold of the flavor compound that contributes the most to overall flavor, respectively. Every contributes compounds satisfies $0 < \mathrm{ROAV} \leq 100$, and the larger the ROAV, the greater the contribution of the compound to the overall flavor of the samples. Compounds with ROAV ≥ 1 are regarded as key volatile flavor compounds capable of greatly affecting the overall flavor of meat, whereas compounds with $0.1 \leq \mathrm{ROAV} < 1$ are thought to exert a moderating impact (Bi et al., 2022).

2.6. Statistical analyses

In the present study, all experimental data were presented as the mean ± standard error (SE). One-way analysis of variance (ANOVA) was conducted applying IBM SPSS 22 (SPSS Inc., Chicago, IL, USA), and Tukey's test was used to examined the differences among groups at a significance level of $p < 0.05$. Spearman correlation analysis was performed in Origin 2021 software (Origin Lab Corporation, Northampton, Massachusetts, USA).

Table 2

Effect of diet supplemented with DOL on physical parameters and chemical composition of broiler breast meat.

Item	Dose of *D. officinale* leaves in Diet			SEM	*p*-Value
	0%, CON	1%, DL1	5%, DL2		
Physical parameters[1]					
Shear force (kgf/cm)	2.24^{a}	1.59^{b}	1.48^{b}	0.072	< 0.001
Cooking loss (%)	25.38^{b}	26.31^{b}	28.99^{a}	0.533	0.007
pH$_{15}$	5.96^{b}	6.17^{a}	6.10^{a}	0.022	< 0.001
*L** (15 min)	48.38	47.85	47.95	0.773	0.960
*a** (15 min)	3.55	3.73	3.96	0.184	0.668
*b** (15 min)	13.98^{a}	12.14^{ab}	11.58^{b}	0.366	0.015
Chemical composition[2]					
Moisture, %	70.90	71.24	70.40	0.159	0.087
Crude protein, %	20.76	20.62	20.71	0.114	0.888
Crude fat, %	2.81^{b}	2.94^{ab}	2.98^{a}	0.029	0.033
Ash, %	4.24	4.26	4.20	0.031	0.771

[1] $n = 12$ replicates per treatment.

[2] $n = 6$ replicates per treatment; SEM, standard error of the mean; pH$_{15,}$ pH of breast muscles measured fifteen minutes postmortem; *L**, lightness; *a**, redness; *b**, yellowness; ^{ab} Means with different superscripts within the same row indicate statistical differences ($p < 0.05$).

3. Results

3.1. Physical parameters and chemical composition of breast meat

Table 2 presented the physical parameters and chemical composition of broiler meat. Shear force values of breast muscles were significantly reduced in the DOL supplemented groups ($p < 0.05$), from 2.24 kgf/cm in the CON group to 1.59 kgf/cm in DL1 group and 1.48 kgf/cm in DL2 group, whereas, the pH values at 15 min in DL1 group (6.17) and DL2 group (6.10) were significantly higher than that in the CON group (5.96; $p < 0.05$). For meat color, there was a significant decrease ($p < 0.05$) in the b^* value of breast meat in the DL2 group (11.58) compared to that in the control group (13.98), but had no significant differences in L^* and a^* values of breast muscles among groups ($p > 0.05$).

The chemical composition of chicken breast meat was also displayed in Table 2. The data showed that feeding diets supplemented with DOL could increase the crude fat content of breast meat, especially in the 5% addition group, which reached 2.98% and was significantly higher than that in the control group (2.81%; $p < 0.05$). No significant differences in crude protein, moisture, and ash percentages were found among groups ($p > 0.05$).

3.2. Fatty acid composition of the breast meat

The evaluation of the fatty acid profile performed on breast meat was shown in (Table 3). The most represented fatty acids detected in breast muscle was oleic acid (C18:1n-9c), followed by palmitic acid (C16:0) and linoleic acid (C18:2n-6). Data showed that dietary supplementation of DOL resulted in significant changes in the fatty acid profile of chicken breast meat. Compared to the control group, total SFA content was significantly higher in two experimental groups, which was due to a significant increase in stearic acid (C18:0) content ($p < 0.05$). On the contrary, the levels of oleic acid (C18:1n-9c) and total MUFA were significantly reduced after DOL supplementation ($p < 0.05$). Furthermore, significant improvements ($p < 0.05$) in total PUFA content were observed with the inclusion of DOL, from 198.11 g/kg in the control group to 204.67 g/kg in DL1 group, and 207.79 g/kg in DL2 group. Specifically, the concentrations of all detected PUFAs, including α-Linolenic acid (C18:3n-3), docosahexaenoic acid (C22:6n-3), linoleic acid (C18:2n-6), and docosatetraenoic acid (C22:4n-6) were increased in two experimental groups. As a consequence, the ratio of PUFA to MUFA also increased significantly ($p < 0.05$) in DOL-added groups. While the total amounts of n-3 PUFAs and n-6 PUFAs were both markedly higher in two DOL dietary supplementation groups, the n-6/n-3 PUFA ration decreased in the DL1 group ($p < 0.05$). Lastly, no significant treatment effect was observed on the PUFA to SFA ratio ($p > 0.05$).

Table 3
Fatty acid composition and content of chicken breast meat samples[1].

Fatty acid, g/kg of lipids	Dose of *D. officinale* leaves in Diet			SEM	p -Value
	0%, CON	1%, DL1	5%, DL2		
C14:0 (Myristic acid)	4.03	4.07	4.09	0.024	0.572
C16:0 (Palmitic acid)	199.89	208.29	207.45	1.971	0.161
C18:0 (Stearic acid)	89.10^{c}	93.49^{b}	95.56^{a}	0.722	< 0.001
Σ SFA	293.02^{b}	305.85^{a}	307.10^{a}	2.424	0.020
C16:1n-7 (Palmitoleic acid)	24.74	25.08	24.90	0.117	0.531
C18:1n-9c (Oleic acid)	259.06^{a}	249.72^{c}	253.59^{b}	1.146	< 0.001
Σ MUFA	283.80^{a}	274.80^{c}	278.49^{b}	1.096	< 0.001
C18:3n-3 (α-Linolenic acid, ALA)	8.33^{b}	9.02^{a}	9.09^{a}	0.087	< 0.001
C22:6n-3 (Docosahexaenoic acid, DHA)	14.12^{b}	15.04^{a}	14.25^{b}	0.109	< 0.001
n-3 PUFA	22.45^{c}	24.06^{a}	23.34^{b}	0.166	< 0.001
C18:2n-6 (Linoleic acid, LA)	163.83^{b}	168.47^{a}	172.37^{a}	1.139	0.002
C22:4n-6 (Docosatetraenoic acid)	11.84^{b}	12.15^{a}	12.09^{a}	0.048	0.011
n-6 PUFA	175.66^{b}	180.62^{a}	184.45^{a}	1.153	0.002
Σ PUFA	198.11^{b}	204.67^{a}	207.79^{a}	1.227	0.001
Total	774.93^{b}	785.32^{ab}	793.38^{a}	2.658	0.008
n-6/n-3	7.83^{a}	7.51^{b}	7.91^{a}	0.057	0.004
PUFA/MUFA	0.70^{b}	0.75^{a}	0.75^{a}	0.006	< 0.001
PUFA/SFA	0.68	0.67	0.68	0.005	0.852

[a-c] Values with different superscripts in the same row differ significantly ($p < 0.05$).

[1] n = 6 replicates per treatment; SEM, standard error of the mean; SFA, saturated fatty acids (C14:0, C16:0, C18:0); MUFA, monounsaturated fatty acids (C16:1n7, C18:1n9c); PUFA, polyunsaturated fatty acids (C18:2n6c, C18:3n3, C22:4, C22:6n3); PUFA/MUFA, polyunsaturated to monounsaturated fatty acids ratio; PUFA/SFA, polyunsaturated to saturated fatty acids ratio.

3.3. Volatile compounds in chicken meat

The data of all the volatile compounds (VOCs) detected in the breast samples through HS-SPME-GC–MS are listed in supplementary material Table S1. In this study, a total of 239 kinds of volatile compounds (VOC) were detected from all breast muscle samples and categorized into seven chemical classes, including 28 aldehydes, 31 alcohols, 27 ketones, 34 esters, 88 hydrocarbons, 4 furans, and other compounds. The results of the relative peak area percentage of major categories revealed that aldehydes were the most predominant class of volatile compounds detected in chicken breast muscles (44.04% - 54.06% of the total VOC), followed by alcohols (22.92% - 34.22%), and ketones (3.62% - 4.05%), esters, furans, and hydrocarbons were detected in minor amounts (Fig. 1a). There was a negative correlation between the relative content of total aldehydes and alcohols ($r = 0.83$, $p < 0.05$), as well as a negative correlation between the relative content of total alcohols and ketones ($r = 0.68$, $p < 0.05$) (Fig. 1b).

In order to further investigate the differences between control group and DOL treatment group, 44 volatile compounds with relative content >0.1% were selected for statistical analysis, and they represented >95% of the total VOCs (Table 4). Obviously, dietary supplementation with DOL significantly affected the content of several volatile compounds. Hexanal was the most represented compound, which accounted for the >25% of total VOCs in all meat samples. Significant increase in butanal, pentanal, hexanal, heptanal, and total aldehydes relative contents were observed in samples obtained from chicken fed with DOL supplementation ($p < 0.05$). The relative contents of octanal, tetradecanal, and pentadecanal also significantly increased in DL2 group ($p < 0.05$). DOL intake did not cause significant changes in alcohols, but the top three alcohols, ethanol, 1-hexanol, and 1-octen-3-ol dominated a decreasing trend in DOL-treated groups, which resulted in a significant lower relative content of total alcohols in DL2 group ($p < 0.05$). Among the detected ketones, the DL2 samples had the highest contents of 2-heptanone (2.04 ± 0.04%) and 2-butanone (0.38 ± 0.05%). Although more types of hydrocarbons (88) were identified than others, only a few of them had relative content higher than 0.1%, such as toluene and pentyloxirane, which had significantly higher ($p < 0.05$) contents in DL2 group than in the CON group (Table 4). With regard to furans, 2-pentylfuran was the most representative in all samples without significant differences among groups ($p > 0.05$).

3.4. Identification of key aroma component based on ROAV

Through searching, we obtained the aroma thresholds for 25 volatile flavor compounds, and calculated the ROAV values based on the relative content and threshold of each compound, as presented in Table 5. Nine compounds with ROAV value >1 were screened out, consisting of seven

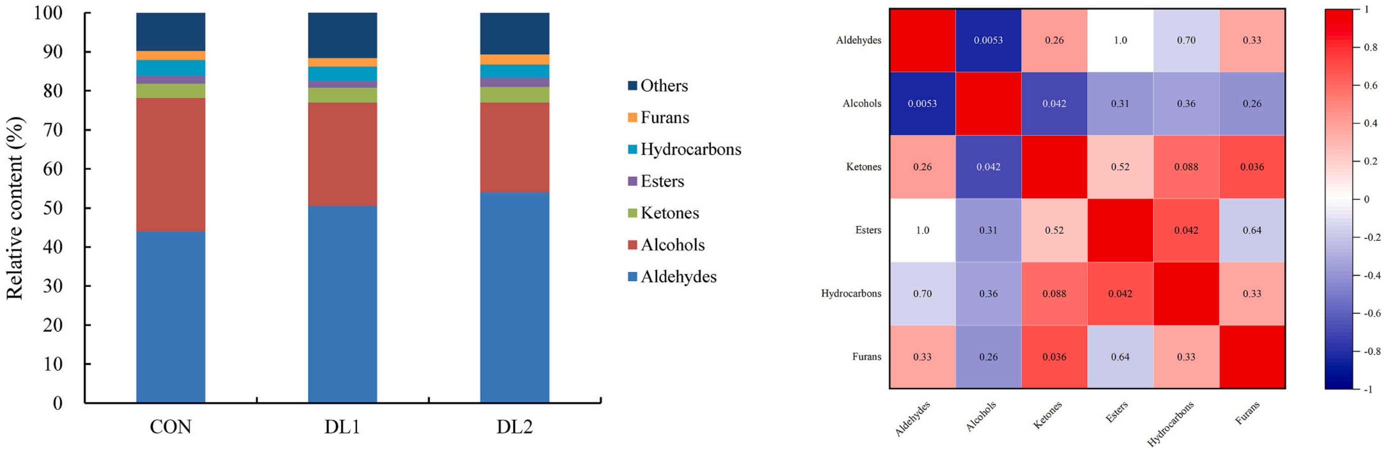

Fig. 1. Analysis of relative content of main volatile categories. A, The relative content of total aldehydes, alcohols, ketones, esters, hydrocarbons and furans in the three groups; B, The correlation analysis of the relative content of total aldehydes, alcohols, ketones, esters, hydrocarbons and furans. CON, DL1, and DL2 represent groups feed diets containing 0%, 1%, and 5% DOL, respectively.

kinds of aldehydes, as well as 1-octen-3-ol and 2-pentylfuran, which were found to be the key flavor compounds of breast meat in three groups. Particularly, 1-octen-3-ol and hexanal, with the highest ROAV (ROAV = 100), were the mostly contribute to the overall flavor of chicken breast muscles in CON and DOL-treatment groups, respectively. Moreover, the ROAV values of the key volatile aroma compounds exhibiting fatty odors (e.g., hexanal, heptanal, octanal, and nonanal) were higher in DL1 and DL2 groups than in CON group. While the ROAV values of (E, E)-2,4-decadienal, 1-octen-3-ol, and 2-pentylfuran decreased in experimental groups, compared to control group.

4. Discussion

We have previously studied the effects of *D. officinale* leaves used as a feed additive on the growth performance and health status of broiler chickens (Zhao et al., 2023), but meat quality, a major concern for consumers, can be affected by the nutritional and healthy status of animal (Mendonca et al., 2020). Therefore, it is necessary to figure out whether there are alterations in meat quality after dietary DOL addition. The current study was conducted with the aim to evaluate the effects of two different dietary DOL supplementation (1% and 5, respectively) on physical parameters, chemical composition, fatty acid profile, and volatile compound of broiler breast meat.

4.1. Physical and chemical characterization of chicken breast meat

Shear force, pH, and meat color are the main physical indices used to assess the meat quality of livestock and poultry. The shear force is considered as a basic indicator of meat tenderness, and the higher the tenderness, the lower the shear force. In this study, significant decreased shear force values were observed in breast meat of DOL addition broilers, suggesting that dietary DOL supplementation had beneficial effects on improving chicken meat tenderness.

Previous study demonstrated that the accumulation of lactic acid in meat due to glycolysis in the postmortem period led to a significant drop in pH and a decrease in the final pH, resulting in muscle protein denaturation and meat quality deterioration (Meng et al., 2020). Thus, the pH value is a crucial indicator for evaluating meat quality. In the current study, the pH values at 15 min were significantly increased in the breast muscles of broilers in the two DOL-treated groups than the control group, indicating that dietary DOL supplementation could inhibit the reduction in pH values and helped to maintain the meat quality. There is little literature on the effects of DOL on meat pH, but several studies reported that supplementing the diet with antioxidants like resveratrol (Jin et al., 2021) could reduce the lactate dehydrogenase activity and the anaerobic glycolysis process in muscle, leading to a decrease of lactic acid content, a slower rate of the pH decline, and an increase of final pH value, which was associated with the up-regulated antioxidant capacity of animals.

Meat color is considered a determinant of meat quality and freshness, directly affecting the acceptance and purchase intention of consumers for meat products. The present study showed that the lightness (L^*) and redness (a^*) parameters of breast muscles did not differ significantly among groups, nevertheless a^* showed an increasing trend and L^* was slightly decreased in two experimental groups. However, compared to the control group, broilers fed 5% DOL had a significant lower yellowness (b^*) value. Myoglobin can be converted to oxygenated myoglobin, and their ratio can directly affect a^* value, while b^* value is an indicator reflecting the content of myoglobin that has been oxidized to high iron myoglobin (Ma et al., 2021). Therefore, changes of meat color may be closely related to the oxidative stress and antioxidation. For example, Jin et al. (Jin, Pang, et al., 2021) reported that dietary resveratrol could increase a^* value and decrease b^* value of meat, which was probably due to the enhancement of the antioxidant enzyme activities induced by resveratrol. We have previously revealed that supplementation DOL to the diet significantly increased the antioxidant capacity of broilers, and this might be responsible for the increase of pH values and improvement (increased a^* value and decreased b^* value) of meat color (Zhao et al., 2023).

The most straightforward way to assess the nutritional value of meat products is to determine their chemical composition. In the present study, dietary supplementation with DOL had no effect on the content of moisture, crude protein, and ash in chicken breast muscles ($p > 0.05$), but increased the content of fat ($p < 0.05$), compared to the control group. Several recent studies have also found no significant differences in meat chemical composition when adding different dietary plants or extracts in the broiler diets, such as roselle extract (Amer et al., 2022) and grape pomace (Bennato et al., 2020). This was the first research on the effect of DOL on the chemical composition in the breast muscles of chickens.

4.2. Meat fatty acid composition

From the standpoint of human health, chicken meat is regarded as a high-quality source of necessary fatty acids and protein. The tenderness, flavor, and nutritional value of meat can be affected by the fatty acid composition, which is a crucial indicator for assessing the meat quality and is greatly influenced by diet manipulation. In the present study, the predominant fatty acids detected in broiler breast meat were C18:1n-9c, C16:0, and C18:2n-6, which together accounted for approximately 80%

Table 4

Effects of dietary DOL on the volatile compounds of boiled breast meat samples.

R.T. (mins)	Compounds	Relative content (%)[1]			Similarity	Odor Description[2]
		CON	DL1	DL2		
Aldehydes						
2.42	Butanal	0.08 ± 0.00^{b}	0.10 ± 0.01^{a}	0.11 ± 0.00^{a}	851	Green, pungent
3.83	Pentanal	8.05 ± 0.52^{b}	9.78 ± 0.06^{a}	10.26 ± 0.12^{a}	902	Green, floral, burning
6.45	Hexanal	25.93 ± 1.16^{b}	30.61 ± 0.45^{a}	31.49 ± 0.83^{a}	922	Green, grassy, fat
9.30	Heptanal	2.67 ± 0.09^{b}	3.32 ± 0.19^{a}	3.82 ± 0.17^{a}	923	Fresh, burnt fat
12.40	Octanal	1.60 ± 0.01^{b}	1.98 ± 0.16^{ab}	2.39 ± 0.19^{a}	940	Fatty, green
13.31	2-Heptenal, (*Z*)-	0.13 ± 0.02	0.13 ± 0.01	0.13 ± 0.01	873	Medicinal
13.62	2-Hexenal, 2-ethyl-	0.38 ± 0.02^{ab}	0.31 ± 0.06^{b}	0.46 ± 0.02^{a}	803	
15.34	Nonanal	1.40 ± 0.08	1.91 ± 0.33	2.14 ± 0.30	945	Fatty, green
18.09	Decanal	0.14 ± 0.05	0.15 ± 0.03	0.14 ± 0.01	833	Green, onion, yeast
18.44	Benzaldehyde	2.53 ± 1.54	1.26 ± 0.03	1.68 ± 0.11	897	Nutty, bitter almond, burnt sugar
25.24	2,4-Decadienal, (E, *E*)-	0.17 ± 0.05	0.15 ± 0.02	0.14 ± 0.01	698	Fatty, toasted, scallion
27.72	Tetradecanal	0.12 ± 0.00^{b}	0.10 ± 0.00^{b}	0.18 ± 0.01^{a}	897	
29.83	Pentadecanal-	0.33 ± 0.02^{b}	0.26 ± 0.01^{b}	0.46 ± 0.02^{a}	936	Fresh
	Subtotal	43.53 ± 1.18^{b}	50.06 ± 0.97^{a}	53.41 ± 0.97^{a}		
Alcohols						
3.15	Ethanol	11.32 ± 6.45	7.31 ± 1.80	6.72 ± 1.17	930	Alcoholic, strong
8.90	1-Penten-3-ol	0.61 ± 0.09	0.71 ± 0.07	0.67 ± 0.02	904	Burnt, green
11.53	1-Pentanol	3.53 ± 0.37	3.85 ± 0.15	3.65 ± 0.05	934	Pungent, fermented
14.43	1-Hexanol	9.00 ± 5.14	4.49 ± 0.42	2.29 ± 0.23	883	Woody, fusel, oily
17.00	1-Octen-3-ol	6.00 ± 0.71	6.09 ± 0.45	5.64 ± 0.10	949	Mushroom, fatty
17.15	1-Heptanol	1.36 ± 0.08	1.59 ± 0.09	1.59 ± 0.11	819	Musty, leafy, violet
19.72	1-Octanol	0.91 ± 0.06	1.04 ± 0.08	1.08 ± 0.10	861	Waxy, green, orange
21.05	2-Octen-1-ol, (E)-	0.45 ± 0.10	0.42 ± 0.06	0.36 ± 0.01	846	Green, citrus, vegetable
21.58	6-Undecanol	0.43 ± 0.10	0.37 ± 0.07	0.31 ± 0.01	774	
	Subtotal	33.61 ± 3.79^{a}	25.87 ± 1.32^{ab}	22.33 ± 0.99^{b}		
Ketones						
2.66	2-Butanone	0.17 ± 0.01^{b}	0.23 ± 0.01^{b}	0.38 ± 0.05^{a}	890	Spicy
5.78	2,3-Pentanedione	0.08 ± 0.01	0.11 ± 0.00	0.11 ± 0.01	827	Almond, burnt, butter
9.26	2-Heptanone	1.71 ± 0.05^{b}	1.85 ± 0.04^{b}	2.04 ± 0.04^{a}	918	Fruity, almond
10.92	2-Heptanone, 6-methyl-	0.36 ± 0.02	0.35 ± 0.01	0.38 ± 0.02	870	Fruity
11.41	3-Octanone	0.15 ± 0.03	0.13 ± 0.01	0.12 ± 0.00	875	Earthy, mushroom
12.21	Acetoin	0.14 ± 0.08	0.23 ± 0.13	0.09 ± 0.02	867	Cream
12.31	2-Octanone	0.19 ± 0.03	0.16 ± 0.01	0.16 ± 0.00	815	Floral, fruity
12.75	1-Octen-3-one	0.16 ± 0.04	0.14 ± 0.01	0.14 ± 0.02	633	Metallic, mushroom
15.79	5-Ethylcyclopent-1-enecarboxaldehyde	0.26 ± 0.05	0.25 ± 0.02	0.25 ± 0.01	805	
	Subtotal	3.24 ± 0.19	3.44 ± 0.19	3.66 ± 0.14		
Esters						
13.57	n-Caproic acid vinyl ester	1.24 ± 0.10	0.10 ± 0.50	1.67 ± 0.45	813	
27.67	Tributyl phosphate	0.14 ± 0.02^{b}	0.20 ± 0.01^{a}	0.14 ± 0.02^{b}	681	
38.72	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.14 ± 0.03	0.15 ± 0.02	0.14 ± 0.03	937	
41.12	Dibutyl phthalate	0.20 ± 0.03	0.24 ± 0.04	0.22 ± 0.04	939	
	Subtotal	1.72 ± 0.03	1.58 ± 0.56	2.19 ± 0.49		
Hydrocarbons						
5.16	Toluene	0.14 ± 0.00^{b}	0.17 ± 0.02^{ab}	0.20 ± 0.01^{a}	910	Nutty
8.05	Oxirane, pentyl-	0.17 ± 0.01^{b}	0.22 ± 0.01^{ab}	0.25 ± 0.02^{a}	888	
23.11	Heptadecane	1.02 ± 0.69	0.79 ± 0.68	0.79 ± 0.55	868	Alkane
24.61	4-Decene, 2,2-dimethyl-, (*E*)-	0.12 ± 0.03	0.13 ± 0.01	0.12 ± 0.01	727	
	Subtotal	1.46 ± 0.71	1.30 ± 0.68	1.35 ± 0.55		
Furans						
3.38	Furan, 2-ethyl-	0.09 ± 0.02	0.10 ± 0.01	0.12 ± 0.01	885	Chemical, beany, nutty
7.66	2-n-Butyl furan	0.25 ± 0.01	0.27 ± 0.02	0.28 ± 0.00	825	
10.68	Furan, 2-pentyl-	1.96 ± 0.21	1.81 ± 0.13	2.17 ± 0.15	960	Bean, nutty
	Subtotal	2.30 ± 0.24	2.18 ± 0.16	2.58 ± 0.16		
Others						
13.48	Pentanoic acid, 2-methyl-, anhydride	8.81 ± 0.87	10.52 ± 1.15	9.53 ± 0.19	845	
26.25	Hexanoic acid	0.44 ± 0.10	0.57 ± 0.05	0.57 ± 0.19	742	Sweat, cheese
	Subtotal	9.25 ± 0.95	11.09 ± 1.12	10.10 ± 0.34		
	Total	95.101 ± 1.46	95.53 ± 1.05	95.61 ± 0.61		

[1] Data are represented as Mean ± SEM; ^{ab} Different letters in the same row indicate significant difference ($p < 0.05$).

[2] The odor descriptions of volatile compounds referred to (He et al., 2022; Liu et al., 2022; Liu et al., 2023); CON, DL1, and DL2 represent groups feed diets containing 0%, 1%, and 5% DOL, respectively.

Table 5
The ROAV of volatile flavor compounds in breast muscles of chicken.

Compound	Odor threshold (μg/kg)[1]	ROAVs			Odor description
		CON	DL1	DL2	
Pentanal	12	11.17	11.98	12.22	Green, floral, burning
Hexanal	4.5	96	100	100	Green, grassy, fat
Heptanal	3	14.84	16.28	18.21	Fresh, burnt fat
Octanal	0.70	38.19	41.49	48.69	Fatty, green
2-Heptenal, (*Z*)-	13.5	0.16	0.14	0.14	Medicinal
Nonanal	1	23.28	28.08	30.62	Fatty, green
Decanal	2	1.17	1.08	1.01	Green, onion, yeast
Benzaldehyde	350	0.12	0.05	0.07	Nutty, bitter almond
2,4-Decadienal, (E, *E*)-	0.07	41.56	31.05	27.83	Fatty, toasted, scallion
Ethanol	100,000	< 0.01	< 0.01	< 0.01	Alcoholic, strong
1-Penten-3-ol	400	0.03	0.03	0.02	Burnt, green
1-Pentanol	150	0.39	0.38	0.35	Pungent, fermented
1-Hexanol	500	0.30	0.13	0.07	Woody, fusel, oily
1-Octen-3-ol	1	100	89.48	80.55	Mushroom
1-Heptanol	425	0.05	0.06	0.05	Musty, leafy, violet
1-Octanol	110	0.14	0.14	0.14	Waxy, green, orange
2-Octen-1-ol, (E)-	20	0.37	0.31	0.26	Green, citrus, vegetable
2-Heptanone	140	0.20	0.19	0.21	Fruity, almond
3-Octanone	28	0.09	0.07	0.06	Earthy, mushroom
2-Octanone	50	0.06	0.05	0.05	Floral, fruity
1-Octen-3-one	5	0.55	0.41	0.39	Metallic, mushroom
Furan, 2-ethyl-	2.3	0.68	0.66	0.77	Chemical, beany, nutty
2-n-Butyl furan	5	0.82	0.79	0.80	
Furan, 2-pentyl-	5.8	5.63	4.59	5.36	Bean, nutty
Hexanoic acid	1840	< 0.01	< 0.01	< 0.01	Sweat, cheese

[1] Threshold values in water of volatile compounds referred to (Yuan et al., 2022; Zhu, Chen, Wang, Niu, & Xiao, 2017); CON, DL1, and DL2 represent groups feed diets containing 0%, 1%, and 5% DOL, respectively.

of the total fatty acids. Dietary SFA, especially stearic, myristic and palmitic acid, are involved in the occurrence of coronary heart disease because of their high cholesterol properties (Wood et al., 2008). Our results found that DOL supplementation increased the total SFA content, especially the C18:0. Although some researches have revealed that low levels of SFA in the human diet may help prevent chronic diseases, others have reported no association between SFA intake and the development of cardiovascular disease (Chowdhury et al., 2014). Furthermore, the PUFA/SFA ratio, a commonly used indicator for evaluating the impact of food on cardiovascular health, did not show any change in our study. Notably, stearic acid (C18:0) and SFA contents significantly increased along with decreases in oleic acid (C18:1n-9c) and MUFA concentrations, and similar changes were also reported by de Souza Vilela et al. (de Souza Vilela et al., 2021). We speculated that this might be due to the reduced conversion efficiency of stearic acid (C18:0) to oleic acid (C18:1n-9c) resulted from DOL supplementation, but the mechanism is not fully understood.

On the contrary, PUFAs are considered to be important for human body, and consuming a certain amount of UFAs has many benefits for human health, such as cancer risk reduction, cardiovascular protection, and brain function improvement (Zarate et al., 2017). Particularly, a higher dietary intake of n-3 PUFA has been shown to contribute to anti-inflammatory and cardiovascular disease control in humans (Zarate et al., 2017), and thus the increase of n-3 PUFA content more specifically represents an improvement in health indicators of food products. In our research, significant higher C18:3n-3 (ALA), C22:6n-3 (DHA), as well as total n-3 PUFA contents were observed in meat samples obtained from chickens in DOL supplementation groups compared to control group, suggesting that DOL had the potential to enrich meat with n-3 PUFA. The n-6 PUFA such as linoleic acid (LA, C18:2n-6), which has positive effects on lowering serum cholesterol concentration and preventing cardiovascular disease (Zarate et al., 2017), was also significantly elevated in the DOL-treatment groups. Furthermore, higher n-6/n-3 PUFA ratios in human diets are known to be detrimental to human health, and meat products with enriched n-3 PUFA or a balanced n-6/n-3 ratio are more popular among consumers. Herein, the n-6/n-3 PUFA ratio in the breast muscle markedly lowered in response to feeding with addition of 1% DOL (DL1). Taken together, these findings indicated that supplementing DOL to broiler diet could improve meat nutritional value by beneficially affecting the fatty acids profile in breast meat. The reason for this might be that the components with antioxidant activity in DOL reduced the oxidation of fatty acids in breast muscle by preventing PUFA oxidation, and similar effects have also been reported for other additives with antioxidant activity (Kafantaris et al., 2018).

4.3. Volatile compounds profile of chicken breast meat

Consumer acceptance of meat products is also influenced by meat aroma, which serves as a crucial indicator of meat quality. The effect of volatile compounds on meat flavor has been widely studied. There are various factors influencing the VOC accumulation in animal tissues, among which animal diet plays a pivotal role. In this study, according to GC–MS analysis, the addition of DOL changed the composition and proportion of the volatile compounds in chicken breast muscles. Such variations in volatile profiles in animal products caused by changes in feeding strategies, particularly the supplemented of vegetable matrices rich in bioactive compounds, have been reported previously (Bennato et al., 2020). The volatile compounds we detected included several of the most common classifications, such as aldehydes, alcohols, ketones, and esters, with aldehydes being the most abundant, especially hexanal, which was detected in the highest quantities, similar to what was previously reported for chicken meat samples (Bennato et al., 2020). Literatures have documented many complex relationships among VOCs, and our finding of the negative correlation between the contents of total aldehydes and alcohols was consistent with previous study (Jin, Cui, et al., 2021).

Aldehydes are key flavor compounds produced by amino-acid Strecker reaction and oxidative degradation of fatty acids, and have a considerable impact on meat flavor owing to their low odor perception thresholds (Elmore et al., 2005). Higher total aldehydes amounts were observed in DOL-treated groups than in the control group ($p < 0.05$). Notably, there was a prominent increase in the relative content of hexanal, which is mainly derived from the oxidation of linoleic acid, and has a 'green/grass/fatty' odor (Marco, Navarro, & Flores, 2006). Additionally, compared with the control group, DOL groups also showed significant higher amounts of pentanal (green) and heptanal (fatty), the oxidation products of linolenic acid and arachidonic acid, respectively (Del Pulgar et al., 2011). Nonanal is a PUFA derived lipid peroxidation product (Ortuno, Serrano, & Banon, 2016), which exhibited an increase trend in the DOL groups. Therefore, it may be speculated that the elevated contents of these aldehydes in the breast muscles from chicken supplemented with DOL were consequences of the increased concentrations of fatty acids, particularly PUFAs, which are susceptible to lipid oxidation owing to the presence of double bonds.

The contents of volatile compounds are not directly correlated with flavor profile. Not all volatile compounds contribute to the flavor of meat, which is only affected when the compound reaches a certain threshold. Therefore, the contribution of volatile flavor compounds to the meat overall aroma is considered to be determined by the concentration and threshold (Zhang et al., 2020). ROAV, the ratio of the content to the odor threshold of the volatile compound, was used in the contribution evaluation of component to the overall flavor. A higher ROAV indicates a greater contribution of the compound to meat flavor. It was worth noting that the volatile compounds with ROAV above 1, hexanal, octanal, nonanal, (E, E)-2,4-decadienal, decanal, heptanal, and pentanal were identified as the key flavor compounds, of which, except for (E, E)-2,4-decadienal and decanal, all displayed higher ROAV values in DL1 and DL2 groups than in the CON group. The results suggested that DOL treatment might affect the flavor of breast meat by affecting the contents of volatile compounds.

Alcohols were the second most abundant VOC detected in the present study, which are also formed from fatty acids oxidation (Campo et al., 2003), but did not change significantly ($p > 0.05$). Among them, ethanol, 1-octen-3-ol, 1-hexanol, and 1-pentanol were the majority of the identified alcohols. It is believed that C18:3n-3 and C18:2n-6 are the major sources of 1-octen-3-ol (Mezgebo et al., 2017). The meat from chickens supplemented with DOL had higher levels of these two fatty acids, but there was no difference in the relative content of 1-octen-3-ol between the groups. This was in line with earlier studies showing that higher levels of C18:3n-3 content do not always translate into higher levels of 1-octen-3-ol in meat and that the formation of alcohols is not solely dependent on the precursors in meat (Mezgebo et al., 2017). Usually, alcohols are considered to have less influence on the meat flavor since their high odor threshold values, while unsaturated alcohols, like 1-octen-3-ol, with a lower threshold value compared to other alcohols, greatly contributes to a typical ‘mushroom’ odor and fatty characteristics in meat (Gkarane et al., 2018). It has been recently reported that 1-octen-3-ol is believed to be one of the very important volatile compounds in Chinese local chicken meat (Jin, Cui, et al., 2021), and this was evidenced by the high ROAV value (> 80) in this experiment again, indicating its key contribution to the meat flavor. The ROAV values of other alcohols, such as 1-hexanol, 1-pentanol, and (E)-2-octen-1-ol were between 0.1 and 1, suggesting their important flavor modification effects in cooked chicken breast meat.

Ketones are usually produced by fatty acid breakdown, the Maillard process, and amino acid breakdown (Mezgebo et al., 2017). We found the primary ketones in chicken meat were 2-heptanone, 2-octanone, 6-methyl-2-heptanone, 1-octen-3-one, and 2-butanone. Consistent with the higher fatty acid content determined in the DOL treatment groups, the relative contents of 2-butanone and 2-heptanone were significant higher ($p < 0.05$) in DL2 group than those in CON group. 2-heptanone has banana-like, cheesy, and slightly medicinal aroma, and it was identified as an important favor-modifying compound in the present study since its ROAV value >0.1.

Furans are vital heterocyclic compounds in meat flavor, and in our study three kinds of furans were detected, including 2-ethylfuran, 2-n-butylfuran, and 2-pentylfuran, with litter difference among groups. According to the ROAV analysis results, 2-pentylfuran was one of the key flavor compounds, which contributed to a nutty flavor, and exists widely in chicken meat (Liu et al., 2023).

Hydrocarbons and esters might contribute trivial to meat flavor given their low concentrations and high thresholds (Ba et al., 2019). Therefore, no further consideration was done on the effects of these compounds on flavor characteristics of chicken meat.

Overall, the results of volatile compound analysis revealed that dietary supplementation with DOL might improve the aromatic profile of chicken meat through increasing the contents of a variety of fatty acids-derived volatile compounds, particularly aldehydes, many of which were identified as key flavor compounds in chicken breast meat.

5. Conclusion

According to the results of current study, the dietary supplementation of *D. officinale* leaves at the tested levels (1% and 5%) was effective in improving the quality of chicken meat, including significantly increasing the pH value, tenderness and intramuscular fat content of breast muscle, as well as the content of PUFAs such as ALA and LA, which is potentially beneficial to consumers’ health. Furthermore, DOL intake contributed to the improvement of the volatile flavor profile of breast meat, especially the content of aldehydes was significantly enhanced.

Ethics statement

The animal study was reviewed and approved by the animal Care and Use Committee of Zhejiang Academy of Agricultural Sciences (Hangzhou, China).

Funding

This work was supported by the Key Research and Development Program of Zhejiang Province (grant number 2024C02004) and Scientific and Technological Inovation 2030-Major Projects (grant number 2023ZD0406401).

CRediT authorship contribution statement

Wanqiu Zhao: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Yong Tian:** Methodology, Investigation, Data curation. **Yunzhu Wang:** Formal analysis. **Jianke Du:** Software. **Li Chen:** Funding acquisition. **Tiantian Gu:** Funding acquisition. **Minquan Song:** Resources. **Lizhi Lu:** Project administration, Conceptualization. **Chongbo Sun:** Writing – review & editing, Conceptualization.

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

The datasets generated for this study are available on request on the ponding author.

Acknowledgements

The authors acknowledge the Zhejiang Xianju breeding chicken farm for providing the experimental house, and the graduate students at Laboratory for Poultry Breeding Research, Institute of Animal Husbandry and Veterinary Medicine, Zhejiang Academy of Agricultural Sciences for supporting assistance in sampling work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101330.

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