Food Chemistry Advances 1 (2022) 100098

Contents lists available at ScienceDirect

Food Chemistry Advances

journal homepage: www.elsevier.com/locate/focha

The volatile compound profile in the meat of chickens raised in a free-range system varies with sexual maturity

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ARTICLE INFO

Keywords:
Taste
Chemical characterization
Meat quality

ABSTRACT

This study aimed to characterize and evaluate the profile of volatile compounds found in the meat of chickens raised in a free-range system as a function of their sex and sexual maturity. A completely randomized design (CRD) was used, and 90 birds were categorized as immature males, mature males, immature females or mature females. Samples of breast and drumstick cuts were analyzed. The volatile compounds were extracted by solid-phase microextraction (SPME), and the compounds were identified by mass spectrometry and the Kovats index. Ninety-six volatile compounds were identified. Aldehydes, terpenes, and alcohols composed the highest numbers of identified volatile compounds. There was a stronger relationship between aldehyde, ketone, terpene, furan and sulfur compounds with drumstick meat from mature males compared with the other samples. Ester compounds were more strongly associated with chicken meat from immature females in breast cut. In breast meat, the alcohol group was negatively correlated with immature chickens in both sexes and increased with sexual maturity. Compounds such as aldehydes, hydrocarbons, carboxylic acids, and pyrazines were more strongly related to the breast meat of mature females. There was a difference in the profile of volatile compounds between mature and immature chickens according to sex and cut. Male and female chickens demonstrated compounds that could change the taste of chicken meat, indicating that the influence of sexual maturity was a common factor.

1. Introduction

Meat taste is one of the characteristics involved in consumers' acceptance of a product. Raw meat tastes similar to blood, with little or no flavor. However, the flavor and taste of cooked meat is due to volatile compounds that arise from lipid degradation, the Maillard reaction, and the interaction between these two reactions during cooking (Jayasena, Ahn, Nam & Jo, 2013; Mottram, 1998; Qi et al., 2017). In chicken meat, the main volatile compounds that influence its taste are aliphatic hydrocarbons, aldehydes, alcohols, ketones, esters, carboxylic acids, aromatic hydrocarbons, and oxygenated heterocyclic compounds (Ayseli, Filik & Selli, 2014; Mottram, 1998). Additionally, specific compounds have been highlighted, such as 2-methyl-3-furanthiol (Tang, Jiang, Yuan & Ho, 2012); hexanal; 2-furfurythiol; 2(E)-nonenal; 2,3(E,E)-decadienal; 2.4(E,Z)-decadienal; and 2,4(E,E)-nonadienal, for cooked chicken meat (Abad, 2018).

In chickens raised in a free-range system, some factors, such as slaughter age and sex, can influence chicken meat sensorial quality due to changes in bromatological and fatty acid composition (Cruz & Faria, 2019; Li et al., 2021; Souza, Faria & Bressan, 2012). The main aspect associated with this change in taste and flavor of chicken meat during sexual maturation is that they become more intense (Berri, 2004; Zanusso & Dionello, 2003). Although the formation and profile of volatile compounds are associated with taste, they have not been completely elucidated (Li et al., 2021; Takahashi, 2018).

In Brazil, the age of slaughter for chickens raised in an alternative or free-range system varies between 70 and 120 days (Brasil, 2020); that is, chickens are slaughtered before they reach sexual maturity. In the literature, studies assessing the taste of meat from chickens raised in an alternative system and slaughtered before sexual maturity do not report differences from conventional chicken meat (Fanatico et al., 2007; Silva, Arruda & Gonçalves, 2017; Smith, Northcutt & Steinberg, 2012).

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https://doi.org/10.1016/j.focha.2022.100098
Received 2 April 2022; Received in revised form 25 August 2022; Accepted 30 August 2022
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Table 1
Components and composition of the feed (Initial, Growth I, Growth II and Final) provided to the free-range chickens.

Ingredients (kg)	Initial Feed(1–28 days)	Growth I Feed (29–49 days)	Growth II Feed (50–70 days)	Final Feed (71–180 days)
Corn	64.70	69.10	72.75	73.45
Soybean bran	31.70	27.70	23.85	22.80
Soybean degummed oil	–	–	0.20	0.90
Kaolin	–	–	0.20	0.20
Calcitic lime	0.10	0.20	–	0.15
Premix for free-range chickens[1]	3.50	3.00	3.00	2.50
Calculated Values	**Initial Feed (kg)**	**Growth I Feed (kg)**	**Growth II Feed (kg)**	**Final Feed (kg)**
EM[2] (kcal/kg)	2949.20	2996.13	3047.97	3098.19
CP[3] (%)	20.07	18.50	17.01	16.50
Calcium (%)	1.05	0.9051	0.87	0.79
Available phosphorus (%)	0.41	0.36	0.36	0.31
Methionine + cystine (%)	0.69	0.64	0.64	0.57
Lysine (%)	0.96	0.87	0.78	0.75
Threonine (%)	0.68	0.63	0.57	0.55
Tryptophan (%)	0.23	0.21	0.19	0.18
Choline (mg/kg)	1153.96	1059.73	994.67	945.74
Sodium (mg/kg)	1848.39	1613.59	1611.59	1375.69
Chlorine (mg/kg)	3103.34	2744.98	2746.98	2381.02

[1] Guaranteed levels for premix of free-range chickens: folic acid (min) 23.33 mg/kg, pantothenic acid (min) 333.33 mg/kg, B.H.T. (min.) 500 mg/kg, biotin (min.) 0.5 mg/kg, calcium (min.) 240 g/kg, calcium (max.) 270 g/kg, copper (min.) 333 mg/kg, choline (min.) 6000 mg/kg, iron (min.) 1667 mg/kg, fluorine (max.) 497.8 mg/kg, phosphorus (min.) 51 g/kg, iodine (min.) 28.33 g/kg, lysine (min.) 10 g/kg, manganese (min.) 2333 mg/kg, methionine (min.) 40 g/kg, niacin (min.) 1000 mg/kg, selenium (min.) 10 mg/kg, sodium (min.) 47.28 g/kg, vitamin A (min.) 159 IU/kg, vitamin B1 (min.) 33.33 mg/kg, vitamin B12 (min.) 333.33 mcg/kg, vitamin B2 (min.) 133.33 mg/kg, vitamin B6 (min.) 66.67 mg/kg, vitamin D3 (min.) 50,000 IU/kg, vitamin E (min.) 266,667 IU/kg, vitamin K3 (min.) 53.33 mg/kg, and zinc (min.) 2000 mg/kg (Vaccinar Nutrição Animal, Belo Horizonte, MG, Brazil). [2]metabolizable energy; [3]crude protein.

However, most consumers of free-range chicken meat seek a product with a more intense taste (Kosowska, Majcher & Fortuna, 2017).

Sexual maturity is one factor that may interfere with the composition of volatile compounds in the meat of free-range chickens, especially due to the increase in fat as the animals grow older (Kosowska et al., 2017; Li et al., 2021; Sun et al., 2017), and may alter taste, given that chickens have a more accentuated taste after sexual maturity (Zanusso & Dionello, 2003). Furthermore, another aspect should be considered: the compositions of meat from breast and drumstick cuts are different (Cruz et al., 2017), which can influence the formation of volatile compounds and consequently sensorial characteristics. However, studies assessing the volatile compounds present in the meat of chickens raised in an alternative system are lacking even though there are differences in meat composition between male and female birds due to the effects of hormones and fat content (Gatellier et al., 2007).

This study aimed to characterize and evaluate the profile of volatile compounds present in the meat of chickens raised in a free-range system as a function of sexual maturity and sex.

2. Material and methods

2.1. Animals and management conditions

One-day-old Label Rouge – Pescoço Pelado chickens were acquired from a commercial hatchery (Globoaves®) and were reared in a conventional broiler house until 28 days of age, comprising a single batch. During this period, the broilers received a basal diet (initial feed) that was formulated to meet their nutritional needs (Table 1). On the 29th day of age, they were relocated to a proper area for raising free-range chickens following the requirements for free-range chicken production in Brazil (ABNT, 2015). Males and females were kept at a stocking rate of 3 m^2 per animal. The area was covered by Tifton grass (*Cynodon* spp. and *Rynchospora* spp.), and the shelter floor was littered with rice hulls at a depth of 10 cm. The birds were fed ad libitum, and their diet consisted of four formulations, according to their growth phase (Table 1). The birds were vaccinated against Marek's disease from a commercial hatchery; Gumboro disease (Hipragumboro® CH80) at 14 days of age, and Newcastle disease and infectious bronchitis (Hipraviar® B1/H120) at 30 days of age, for which both vaccines were administrated through the ocular route at one drop (0.03 ml) per bird (Hipra Saúde Animal Ltda., Porto Alegre, RS, Brazil); and Yaws at 21 days of age in an intradermal wing web with the use of a specific applicator (Biovet, Vargem Grande Paulista, SP, Brazil), according to the vaccination calendar recommend by Globoaves (2015). Deworming occurred at 49 days of age and again at 120 days with the use of fenbendazole vermifuge (Provermin®, Indubras Indústria Veterinária S/A, Contagem, MG, Brazil), which was included at a proportion of 2 g per 20 kg in the feed and supplied for 3 days at a dose of 1 g per 8 kg live weight. Chickens were raised for 180 days in total.

2.2. Slaughter and evaluation of sexual maturity

In this study, a total of 90 chickens (45 males and 45 females) of the Label Rouge (*Pescoço Pelado*) strain were included and slaughtered at 70, 90, 120, 150, or 180 days of age, and each replicate had three birds (one replicate consisted of the mean of the parameters evaluated in three birds slaughtered at each age). The chickens were fasted for 12 h and then slaughtered in accordance with humanitarian methods and recommended manufacturer methods: electrical stunning, bleeding, feather removal, and evisceration. After evisceration, the carcasses were wrapped, labeled, and cooled in a cool chamber, where they remained for at least three hours while the carcass temperature dropped to 5 °C. The cuts (breast and drumstick) were deboned 24 h postmortem, wrapped, labeled, and preserved at −18 °C until being analyzed.

During slaughter, the gonads (testicles and ovaries) were collected to carry out macroscopic and microscopic evaluations and determine the sexual maturity of each chicken. For microscopic evaluation, the samples were previously fixed in 10% formalin, dehydrated, diaphanized, cut at 6 μm thickness and colored with hematoxylin and eosin (HE) to obtain images. The digitized images were obtained using an image capture and analysis system consisting of a CX31 trinocular microscope (Olympus Optical do Brazil Ltda, São Paulo, SP, Brazil) and camera (SC30 Color CMOS Camera for Light Microscopy, Olympus Optical Ltda., São Paulo, SP, Brazil). The images obtained by this system

Table 2
Number of chickens categorized according to sexual maturity and sex at each slaughter age.

Slaughter ages (days)	Immature		Mature	
	Male (n = 18)	Female (n = 21)	Male (n = 27)	Female (n = 24)
70	9	9	–	–
90	9	9	–	–
120	–	3	9	6
150	–	–	9	9
180	–	–	9	9
Weight (kg)	2.91 ± 0.32	2.64 ± 0.26	4.15 ± 0.48	3.30 ± 0.30

were analyzed with ImageJ software (NIH, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA).

The group of sexually immature birds consisted of chickens that did not have complete gonadal development. The level of sexual maturity in males was determined according to Santos, Murakami, Oliveira and Costa (2012) by measuring on microscopic evaluation the diameter of the seminiferous tubules in their testicles, and birds with a testicular diameter of 119.73 µm were considered sexually mature. For females, sexual maturity was visually determined; those with fully developed follicles were considered mature.

2.3. Determination of the sample

The sample for this study was determined based on gonadal development and included the following four groups: immature males (G1), mature males (G2), immature females (G3), and mature females (G4). The total sample of each group and weight are shown in Table 2.

2.4. Collection and transport of samples

The samples used for evaluating volatile compounds were obtained from the carcasses of chickens after they were cooled for three hours to a final temperature of 5 °C. Samples of muscular tissues from the breasts and drumsticks were collected for chemical analyses. These samples were wrapped, labeled, and conditioned in a thermal box. Later, they were transported to the laboratory for further analyses of the profile of the volatile compounds.

2.5. Determination of the volatile compounds

The breast and drumstick samples for the determination of volatile compounds were wrapped in aluminum foil and cooked on an electric plate (Mega Grill; Britânia, Curitiba, PR, Brazil) at 150 °C for approximately 20 min until they reached an internal temperature of 72 °C. Afterward, the samples were cooled at room temperature and crushed, and 5 g of each sample was placed in a 20-mL vial and subjected to the extraction of volatile compounds (VCs). To isolate VCs, the solid-phase microextraction technique (HS-SPME) was used in the confined space (headspace) between the sample and the sealed vial. A DVB/CAR/PDMS coating (Divinylbenzene/Carboxen/Polydimethylsiloxane, 24 ga, 10 mm (Supelco Inc., Bellefonte, PA, USA)) was used to capture the compounds for automatic sampling following exposure to the headspace of the sample for 45 min with the vial heated at 60 °C in an extractor coupled to the equipment. Before extraction, each vial containing the samples was exposed to the same extraction temperature, with the fiber exposed for 10 min (equilibrium time). Afterward, the fiber was automatically inserted into the chromatograph injector for thermal desorption of the analyte.

The VCs were separated and identified on a gas chromatograph coupled to a mass spectrometer in Shimadzu GC/MS-QP 2010 Plus (Agilent Technologies Inc., Palo Alto, CA, USA). Thermal desorption of the fiber analytics occurred with a GC injector at 250 °C in splitless mode, and the fiber was kept exposed inside the injector for 10 min to eliminate the memory effect. The VCs were separated by a fused silica capillary column (5% diphenyl and 95% polysiloxane, model SLBTM-5MS Supelco (30 m x 0.25 mm I.D. x 0.25 μm)) (Supelco Inc., Bellefonte, PA, USA). The temperature program of the column started at 35 °C for two minutes and then increased to 80 °C at a rate of 2 °C min^{-1}. Next, the temperature was raised to 150 °C with a heating ramp of 4 °C min^{-1} and then to 230 °C at a rate of 8 °C min^{-1}, remaining in the isotherm for 5 min. Helium was used as the carrier gas at a constant pressure with an initial flow rate of 1 mL min^{-1}. The analyzer operated in sweeping mode, monitoring the mass from 35 to 350 m/z.

The compounds were evaluated by integration of the chromatographic peaks and expressed as a percentage area of total compound identified, followed by the identification of their mass spectra against those of the GC/MS spectral library (Whiley 8 and FFNSC 1.2 Libraries), as well as by the Kovats index of the experimental retention time of the compounds; the series of alkanes was identified with a standard (C8 to C24) and with their molecular weight according to the methodology used by Adams (2007).

2.6. Statistical analysis

The mean values obtained from the profile of volatile compounds present in the breast and drumstick cuts from four categories (mature male (G1), immature male (G2), immature female (G3), and mature female (G4)) were evaluated through descriptive data analysis and principal component analysis (PCA) conducted in R® (Team, 2019).

3. Results

Seventeen compounds belonging to the aldehyde group and 25 alcohols were identified in the breast and drumstick samples of the analyzed birds. Hexanal was the main aldehyde, while hexanol and 1-octen-3-ol had the highest averages of the alcohols in both cuts. In general, there was an increase in the percentage of most compounds in the aldehyde, alcohol, terpene, and ketone groups after the chickens reached sexual maturity and a decrease in (E)–2-undecenal for both sexes. After the birds reached sexual maturity, a reduction was observed in both cuts for hexanol, 2-heptanol, 1-hexanol, and (E)–3-methyl-2-hepten-1-ol. However, there was an increase in 1-butanol and 3-methyl in the breast cut and a reduction in the same compounds in the drumstick cut after the chickens reached sexual maturity (Table 3).

Seventeen compounds from the terpene group were identified; 1-heptene and 3-methyl- were reduced in the breast and increased in the drumstick cuts, while in both cuts, there were lower amounts of 1-undecene after the chickens reached sexual maturity (Table 3).

A total of 12 hydrocarbons were identified, with higher percentages of tetradecane, tridecane, and pentadecane observed in breast cuts, while cyclopentane and 1-ethyl-2-methyl- were higher in drumstick cuts, especially from sexually mature chickens. However, in drumstick cuts, the compounds dodecane, tridecane, tetradecane, pentadecane, and hexadecane were reduced after sexual maturity. Additionally, esters were found in lower amounts in both cuts after sexual maturity, except for the compounds pentanoic acid, 2-methyl-, and anhydride, which demonstrated an opposite pattern in the drumstick cut (Table 3).

Table 3
Profile of the volatile compounds found in breast and drumstick cuts from Label Rouge chickens categorized by sex and sexual maturity.

Kovats Index	Volatile compounds	Breast					Drumstick				
		Category				SEM	Category				SEM
		IM	IF	MM	MF		IM	IF	MM	MF	
Aldehydes											
801	Hexanal	3.78	3.47	7.33	5.18	10.065	1.71	6.08	6.34	10.20	1.503
1037	Octanal	0.39	0.23	0.26	6.07	10.522	0.20	0.48	0.28	0.20	0.069
1040	Benzeneacetaldehyde	0.17	–	–	–	00.035	–	–	0.21	0.09	0.036
1058	2-Octenal, (E)-	0.70	0.59	1.05	0.76	00.078	0.77	0.83	2.24	1.34	0.169
1102	Nonanal	3.13	1.32	2.34	2.60	00.424	1.03	3.37	2.65	3.68	0.511
1203	Dodecenal	0.13	0.03	0.07	1.19	00.306	0.04	0.15	0.10	0.16	0.028
1213	2.5-Pctadienal, (E.E)-	0.02	0.01	0.04	0.04	00.009	0.03	0.01	0.35	0.10	0.042
1261	2-Decenal, (E)-	0.27	0.29	0.45	0.52	00.056	0.32	0.33	0.54	0.33	0.038
1285	Hexanal, 2-ethyl-	–	–	–	–	–	0.01	0.02	–	–	0.005
1339	2-Butenal, 2-methyl-	–	–	0.02	–	00.006	–	0.01	–	–	0.002
1364	2-Undecenal, (E)-	0.22	0.16	0.41	0.40	00.041	0.23	0.20	0.09	0.06	0.026
1465	Benzaldehyde, 2-methyl-	0.01	0.01	–	–	00.003	–	0.01	–	–	0.001
1512	Tetradecanal	0.07	0.02	0.09	0.05	00.017	0.05	0.03	0.03	0.08	0.012
1615	Octadecanal	0.11	0.06	0.24	0.16	00.035	0.08	0.06	0.15	0.19	0.017
1810	Tridecanal	0.13	0.14	0.33	0.33	00.038	0.05	0.03	0.11	0.09	0.010
1722	Pentadecanal	–	–	0.01	–	00.002	–	–	–	–	–
1191	4-Decenal, (Z)-	0.05	0.01	0.09	–	00.018	0.02	0.05	0.04	0.08	0.017
Alcohols											
809	2,3-Butanediol	0.02	1.03	0.28	0.37	00.153	0.03	0.23	0.36	–	0.105
875	Hexanol	36.24	33.15	15.27	10.68	30.162	43.25	33.64	9.99	12.36	2.973
901	1-Butanol, 3-methyl-	0.80	0.36	0.45	0.23	00.112	0.14	0.49	0.56	0.66	0.091
905	2-Heptanol	0.08	0.03	–	–	00.015	0.01	0.01	–	–	0.003
944	4-Heptanol, 2.6-dimethyl-	–	0.02	0.17	0.28	00.062	–	–	0.04	0.11	0.030
969	2-Hepten-1-ol, (E)-	0.06	0.09	0.05	0.04	00.018	0.03	–	–	–	0.004
971	Heptanol	1.38	1.79	1.52	1.41	00.115	1.29	1.44	1.15	1.31	0.070
980	1-Octen-3-ol	12.83	11.83	22.11	19.16	10.209	15.38	17.23	24.71	23.94	0.950
997	3-Octanol	0.94	0.79	0.13	0.50	00.143	0.91	0.72	0.05	–	0.114
1000	1-Heptanol, 6-methyl-	0.05	–	0.02	0.10	00.021	0.02	0.01	–	0.02	0.005
1002	1.3-Cyclopentanediol, CIS	1.42	0.71	1.15	0.66	00.199	0.38	1.08	1.01	1.23	0.158
1070	2-Octen-1-ol	2.12	2.07	3.07	2.92	00.192	3.77	2.13	4.25	4.44	0.218
1074	1-Octanol	1.89	2.18	2.05	1.91	00.206	1.86	2.30	1.60	1.94	0.148
1085	9-Decen-1-ol	–	0.03	0.02	–	00.008	0.07	0.03	–	0.03	0.013
1093	2,3-Octanediol	1.17	1.16	1.75	1.22	00.223	0.26	0.99	3.97	4.04	0.393
1112	Phenylethyl alcohol	0.08	0.11	–	–	00.020	–	0.11	–	–	0.014
1147	4-Octanol, 7-methyl-	0.01	–	–	–	00.002	–	–	–	–	–
1169	2-Decen-1-ol	–	0.03	–	0.02	00.008	–	–	–	–	–
1174	1-Hexanol, 3-methyl-	0.73	1.07	0.40	0.66	00.094	0.34	0.80	0.29	0.27	0.067
1266	1-Hexanol, 2-ethyl-	0.31	0.38	0.80	0.64	00.057	0.32	0.42	0.76	0.74	0.044
1281	Hexeb-3-ol	0.05	0.04	0.04	0.06	00.013	0.02	0.08	0.04	0.11	0.014
1305	11-Dodecenol	0.02	–	0.01	0.03	00.008	–	–	–	–	–
1492	1-Tetradecanol	0.02	0.03	0.12	0.14	00.015	0.04	0.05	0.14	0.13	0.015
1717	2-Dodecen-1-ol	0.31	0.21	0.67	0.51	00.078	0.14	0.08	0.24	0.26	0.022
Terpenes											
804	1-Heptene, 3-methyl-	4.14	0.39	0.46	0.08	00.766	0.19	0.28	3.63	0.59	1.046
811	2-Octene, (Z)	0.09	0.09	0.03	2.28	00.608	0.06	0.04	0.03	0.01	0.011
864	O-Oxylene	–	–	0.03	0.08	00.021	–	–	0.01	0.01	0.005
975	1–Heptene, 5-methyl-	0.04	0.05	–	–	00.010	0.02	–	0.05	–	0.010
1003	4-Nonene	0.14	0.02	0.02	0.01	00.025	0.03	0.01	–	0.03	0.009
1013	1-Pentene, 2-methyl-	0.02	0.04	0.15	0.06	00.029	0.11	0.11	0.53	0.33	0.081
1027	1.3-Hexadiene, 3-ethyl-2-methyl-	0.30	0.16	0.03	0.11	00.032	0.26	0.24	0.47	0.09	0.091
1072	Cyclohexene, 4-methyl-1-(1-methylethenyl)-	–	–	–	–	–	–	–	–	0.08	0.014
1090	1-Undecene	1.30	1.20	0.12	0.17	00.186	1.96	1.42	0.06	0.13	0.312
1672	1.11-Dedecadiene		–	–	–	–	–	0.02	–	–	0.003
1157	Thiophene, 2-pentyl-	–	0.01	0.08	0.05	00.021	0.07	0.10	–	–	0.015
1160	1-Octene, 6-methyl-	0.34	0.25	0.49	0.44	00.046	0.30	0.35	0.87	0.58	0.072
1151	2.10-Dodecadiene	–	–	–	–	–	0.02	–	0.01	–	0.004
1484	7-Tetradecene, (Z)-	0.05	–	0.01	0.04	00.009	0.02	0.01	–	–	0.003
1581	6-Tetradecene, (Z)-	0.06	0.03	0.01	0.01	00.008	0.02	0.02	–	–	0.004
1679	6-Hexadecene, (Z)-	0.02	–	–	–	00.004	0.01	–	–	–	0.001
1887	2-Octene, 4-ethyl-	–	–	–	–	–	–	0.01	–	–	0.002
Ketones											
888	2-Heptanone	0.46	0.55	0.70	0.64	00.042	0.65	0.64	0.73	0.59	0.039
914	2.5-Pyrrolidinedione, 1-chloro-	0.02	–	0.01	0.04	00.008	0.07	0.01	0.04	0.07	0.015
983	3-Heptanone, 5-methyl-	5.08	4.69	7.59	5.49	00.556	6.48	6.80	7.57	7.94	0.417
1274	2-Decanone	0.01	0.05	0.01	0.72	00.188	0.12	0.15	0.01	–	0.018

(*continued on next page*)

Table 3 (*continued*)

Kovats Index	Volatile compounds	Breast					Drumstick				
		Category				SEM	Category				SEM
		IM	IF	MM	MF		IM	IF	MM	MF	
1449	5.9-Undecadien-2-one, 6.10-Dimethyl-, (E)-	0.04	–	–	0.01	00.005	–	–	–	–	–
1495	2-Tridecanone	–	–	–	0.03	00.004	–	–	–	0.02	0.005
Hydrocarbons											
824	Hexane, 1-methoxy	–	–	–	–	–	0.06	0.04	–	–	0.011
898	Nonane	0.05	0.02	–	0.02	00.009	0.06	0.03	–	–	0.008
955	Cyclopentane, 1-ethyl-2-methyl-	0.15	0.11	0.27	0.34	00.058	0.29	0.22	1.02	0.57	0.099
1015	Hexane, 1-(methylthio)-	0.07	–	0.04	0.02	00.017	0.05	–	0.09	–	0.027
1098	Undecane	0.02	0.01	0.01	–	00.005	0.05	0.07	0.01	–	0.011
1197	Dodecane	0.06	0.06	0.02	0.05	00.014	0.13	0.17	0.02	0.06	0.019
1293	1-Heptyne	0.01	0.01	0.03	0.05	00.008	0.10	0.07	0.20	0.08	0.023
1298	Tridecane	0.53	0.49	0.86	1.01	00.105	0.84	0.85	0.43	0.75	0.080
1370	Octadecane	–	–	–	0.46	00.123	–	–	0.03	–	0.006
1398	Tetradecane	0.65	0.50	0.52	0.60	00.092	0.65	0.67	0.23	0.35	0.072
1499	Pentadecane	0.48	0.26	0.45	0.64	00.080	0.47	0.30	0.17	0.17	0.043
1600	Hexadecane	0.10	0.07	0.07	0.07	00.013	0.08	0.06	0.02	0.02	0.009
Esters											
842	2-Butenoic acid, ethyl ester, (E)-	0.02	0.04	–	–	00.006	0.01	0.06	–	–	0.008
845	Butanoic acid, 2-methyl, ethyl ester	–	0.02	–	–	00.005	–	–	0.01	0.03	0.006
850	Butanoic acid, 3-methyl-, ethyl ester	0.06	0.36	–	–	00.056	–	0.03	–	–	0.006
882	Formic acid, hexyl ester	–	7.84	0.06	–	10.828	–	–	–	–	–
937	2-Butenoic acid, 2-methyl-, ethyl ester	0.04	0.15	–	–	00.026	0.02	0.03	–	–	0.006
1044	2-Hexenoic acid, ethyl ester	–	0.01	–	–	00.003	–	0.04	–	–	0.007
1047	Nonyl nitrate	0.08	0.07	–	–	00.014	0.06	0.10	0.11	–	0.023
1060	Pentanoic acid, 2-methyl-, anhydride	0.27	0.13	0.15	0.12	00.040	0.10	0.06	0.23	0.23	0.051
1806	Octisalate	0.33	0.37	0.45	0.23	00.110	0.03	0.02	0.05	0.03	0.011
1896	Hexadecanoic acid, ethyl ester	–	0.02	–	–	00.004	–	0.01	–	–	0.001
Pyrazines											
817	Hydrazine, ethyl-	–	–	–	0.18	00.047	–	0.02	–	–	0.004
Sulfur compounds											
961	Trisulfite, dimethyl-	0.16	–	0.05	0.05	00.035	–	–	0.12	–	0.036
Furans											
987	Furan, 2-pentyl-	0.89	0.67	0.70	0.60	00.066	0.87	0.84	1.12	0.76	0.072
1664	2(3H)-Furanone, dihydro-5-(2-octenyl)-, (Z)-	–	0.01	0.01	0.03	00.005	0.01	–	0.02	0.02	0.004
1682	2(3H)-Furanone, 5-heptyldihydro-	0.04	–	–	0.03	00.012	–	0.04	–	–	0.010
Carboxylic acids											
890	Butanoic acid, 2-methyl-	–	–	–	0.02	00.006	–	–	–	–	–
1021	Hexanoic acid	–	–	–	0.17	00.046	–	–	0.22	0.74	0.131
1220	Pentanoic acid, 2-ethyl-	–	–	0.01	0.14	00.037	–	–	0.01	–	0.003

SEM - standard error of the mean; IM – immature male chicken; IF – immature female chicken; MM – mature male chicken; MF – mature female chicken.

Only one compound from the pyrazine group was identified in both cuts: hydrazine, ethyl, which was found in higher amounts in the breast of sexually immature females and in the drumsticks of sexually mature females. Of the sulfur compounds, only trisulfite and dimethyl were observed in higher amounts in the breast of immature males and in the drumstick of sexually mature males. In both cuts, three compounds from the furane group and three from the carboxylic acid group were identified.

The main compound from the furane group was furan-2-pentyl, which was found at a higher percentage in the breast cuts of sexually immature males and in the drumstick cuts of sexually mature males. However, among the carboxylic acids, only a few compounds were found in the meat of sexually mature chickens (Table 3), with the highest occurrence being hexanoic acid. This compound from the carboxylic acid group was found in larger quantities in both cuts in females than in males but was present in higher amounts in sexually mature females than immature females.

The principal component analysis for the group of aldehyde compounds in the breast cut is described in Fig. 1A. The breast cut of sexually mature males (G1) differed from the other groups in terms of hexanal (AL1), 2-octenal (AL4), 2,5-octadienal (AL&0,2-decenal (AL8), 2-butenal, 2-methyl- (AL10), 2-undecenal (AL11), octadecanal (AL14), tridecanal (AL15), and pentadecenal (AL16) along principal component (PC) 1. The breast cuts of sexually immature males (G2) and sexually mature males (G1) had a similar pattern with respect to tetradecenal (AL13) and 4-decenal, (Z)- (AL17) along PC 2. The breast cuts of sexually immature males (G2) and sexually immature females (G3) had a similar pattern with respect to the compound benzaldehyde, 2-methyl- (AL12), as this compound had a negative correlation with PC1. The breast cuts of sexually mature females (G4) differed from the other groups in terms of octanal (AL2) and dodecanal (AL6), as these compounds were negatively correlated with PC2.

Regarding alcohols (Fig. 1B), the breasts of sexually immature females (G3) and sexually immature males were similar regarding the occurrence of hexanol (OL2), 2-hepten-1-ol, (E)-(OL6), 3-octanol (OL9), phenylethyl alcohol (OL16), and 1-hexanol, 3-methyl-(OL19) when evaluating positive values of PC1. Nevertheless, in this same cut, the sexually immature females (G3) differed with respect to 2,3-butanediol (OL1), heptanol (OL7), 1-octanol (OL13) and 9-decen-1-ol (OL14) for positive values of PC2. Breast meat from sexually immature male chickens (G1) differed regarding 1-butanal, 3-methyl- (OL3), 2-heptanol (OL4), 1,3-cyclopenanediol (OL11), 4- octanol, 7-methyl-(OL17) and hexen-3-ol (OL21) for negative values of PC2. Breast meat from sexually mature females (G4) and males (G2) had similar occurrences of 4-heptanol, 2,6-dimethyl- (OL5), 1-octen-3-ol (OL8), 2- octen-1-ol (OL12), 1-hexanol, 2-ethyl-(OL20), 1-tetradecanol (OL23) and 2-dodecen-1-ol (OL25) when evaluating the negative values correlated to PC1.

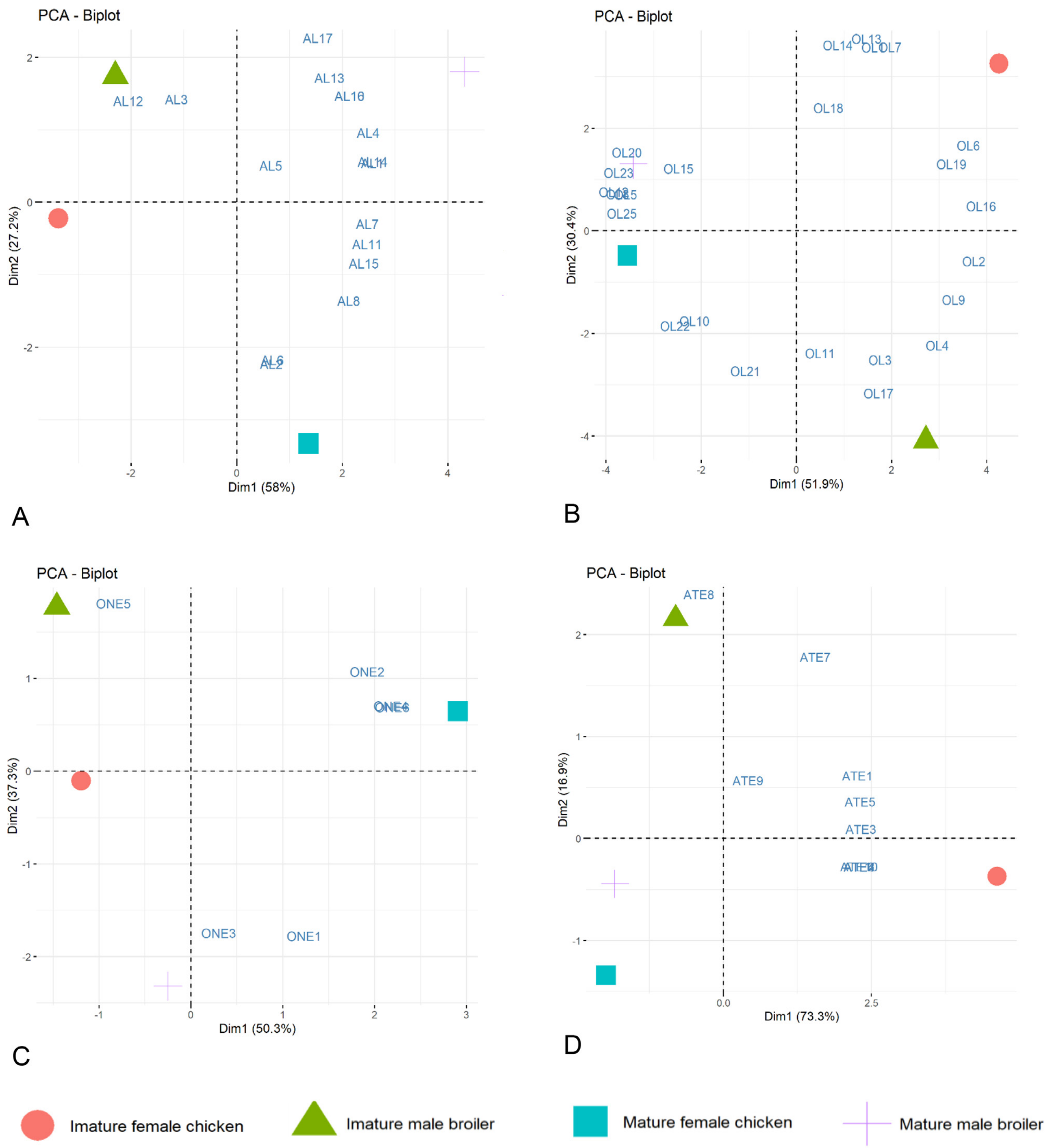

Fig. 1. Principal component analysis of the profile of volatile compounds from the breast cut of chickens raised in a free-range system: (A) aldehyde, (B) alcohols, (C) ketones and (D) esters.

AL1 – hexanal; AL2 – octanal; AL3 – benzene acetaldehyde; AL4–2-octenal, (E)-; AL5 – nonanal; AL6 – dodecanal; AL7–2,5-octadienal, (E,E)-; AL8–2-decenal, (E)-; AL9 – hexanal, 2-ethyl-; AL10–2-butenal, 2-methyl; AL11–2-undecenal, (E)-; AL12 – benzaldehyde, 2-methyl-; AL13 – tetradecanal; AL14 – octadecanal; AL15 – tridecanal; AL16 – pentadecanal; AL17–4-decenal, (Z)-. OL1–2,3-butanediol; OL2 – hexanol; OL3–1-butanol, 3-methyl-; OL4–2-heptanol; OL5–4-heptanol, 2,6-dimethyl-; OL6–2-hepten-1-ol, (E)-; OL7 – heptanol; OL8–1-octen-3-ol; OL9–3-octanol; OL10–1-heptanol, 6-methyl-; OL11–1,3-cyclopenanediol, *cis*; OL12–2-octen-1-ol; OL13–1-octanol; OL14–9-decen-1-ol; OL15–2,3-octanediol; OL16 – phenylethyl alcohol; OL17–4-octanol, 7-methyl-; OL18 – decen-1-decenol; OL19–1-hexanol, 3-methyl-; OL20–1-hexanol, 2-ethyl-; OL21 – hexen-3-ol; OL22–11-dodecenol; OL23–1-tetradecanol; OL24–1-dodecanol; OL25–2-dodecen-1-ol. ONE1–2-heptanone; ONE2–2,5-pyrrolidinedione, 1-chloro-; ONE3–3-heptanone, 5-methyl-; ONE4–2-decanone; ONE5–5,9-undecadien-2-one, 6,10-dimethyl-, (E)-; ONE6–2-tridecanone. ATE1–2-butenoic acid, ethyl ester, (E)-; ATE2 – butanoic acid, 2-methyl-, ethyl ester; ATE3 – butanoic acid, 3-methyl-, ethyl ester; ATE4 – formic acid, hexyl ester; ATE5–2-butenoic acid, 2-methyl-, ethyl ester; ATE6–2-hexenoic acid, ethyl ester; ATE7 – nonyl nitrate; ATE8 – pentanoic acid, 2-methyl-, anhydride; ATE9 – octisalate; ATE10 – hexadecanoic acid, ethyl ester.

The breast meat of sexually immature male and female chickens (G1 and G3, respectively) were similar in terms of 5,9-undecadien-2-one,6,10-dimethyl-(E) (ONE5) and differed with respect to compounds of the ketone group. The breast meat of sexually mature females (G4) was associated with compounds 2,5-pyrrolidinedione, 1-chloro-(ONE2); 2-decanone (ONE4) and 2-tridecanone (ONE6), while sexually mature males (G2) were related to compounds 2-heptanone (ONE1) and 3-heptanone, 5-methyl- (ONE3) (Fig. 1C).

Sexually immature males and females (G1 and G3, respectively) differed with the occurrence esters in breast meat (Fig. 1D). The breast meat of G3 females contained 2-butenoic acid, ethyl ester, (E)- (ATE1); butanoic acid, 2-methyl-, ethyl ester (ATE2); butanoic acid, 3-methyl-, ethyl ester (ATE3); formic acid, hexyl ester (ATE4); 2-butanoic acid, ethyl ester (ATE5); 2-hecenoic acid, ethyl ester (ATE6) and hexadecanoic acid, ethyl ester (ATE10). On the other hand, the breast meat of G1 males contained nonyl nitrate (ATE7) and pentanoic acid, 2-methyl-anhydride (ATE8). The breast meat of sexually mature chickens (G2 and G4) did not show a direct relationship with the ester compounds identified in this work.

There was a divergence between the hydrocarbon compounds present in the breast meat of G4 females (sexually mature) and G1 males (sexually immature) in relation to the other groups (Fig. 2A). The volatile compounds cyclopentane, 1-ethyl-2-methyl- (ANE3), 1-heptyne (ANE7) and tridecane (ANE8) were positively correlated with PC1 and were more closely related to the breast meat of sexually mature female birds (G4). On the other hand, the compounds undecane (ANE5) and hexadecane (ANE12) were strongly negatively correlated with PC1 and were more closely related to the breast meat of sexually immature males (G1). Breast meat from immature females (G3) and mature males (G2) did not show a direct relationship with the compounds of the hydrocarbon group identified in this study (Fig. 2A).

The breast meat of sexually immature males (G1) differed from the other groups in 1-heptene, 3-methyl- (ENE1); 4-nonane (ENE5), 1,3-hexadiene, 3-ethyl-2-methyl-(ENE7); 1-undecene (ENE9); 6-tetradecene (ENE15) and 7-hexadecene (ENE17), which were positively correlated with PC1. The breast meat of sexually immature female chickens (G3) was not related to 1-heptene or 5 methyl- (ANE4) and was negatively correlated with PC2. Breast meat from sexually mature females and males (G2 and G4) was similar in thiophene, 2-pentyl- (ENE11). Furthermore, breast meat from sexually mature females (G4) and immature males was similar with regard to 7-tetradecene (ENE14), which was correlated with PC2 (Fig. 2B).

The breast meat of sexually mature females (G4) differed from the other groups in pyrazine, 2,6 dimethyl- (INE1); 2(3H)-furanone, dihydro-5-(2-octenyl)-,(Z)-(RAN2); butanoic acid, 2-methyl-(OIC1); hexanoic acid (OIC2), and pentanoic acid, 2-ethyl- (OIC3), which were more strongly positively correlated with PC1. The breast meat of immature males (G1) presented the compounds trisulfide, dimethyl- (SUL1); furan, 2-pentyl- (RAN1) and 2(3H)-furanone, 5-heptydihydro- (RAN3), and these compounds were more strongly positively correlated with PC2. The breast meat of sexually immature females (G3) and sexually mature males (G2) was similar in that it was negatively correlated to PC1 and PC2 and showed no relationship with any of the compounds identified in the pyrazine, sulfur compound, furan, and carboxylic acid groups (Fig. 2C).

The volatile compounds identified in the drumstick cuts are displayed in Figs. 3 and 4. The drumstick meat of sexually mature (G3) and immature females (G4) was similar with respect to hexanal (AL1), nonanal (AL5), dodecanal (AL6) and 4-decenal, (Z)- (AL17) and was more strongly positively correlated with PC2. Additionally, the drumstick meat of sexually mature chickens (G1 and G3) was similar with regard to hexanal, 2-ethyl- (AL9) and 2-undecenal, (E)- (AL11), which were negatively correlated with PC1. The drumstick meat of sexually mature birds (G2 and G4) was similar in benzeneacetaldehyde (AL3); 2-octenal, (E)-(AL4); 2,5-octadienal, (E,E)-(AL7); octadecanal (AL14) and tridecanal (AL15) (Fig. 3A).

Regarding the compounds of the alcohol group (Fig. 3B), the drumstick meat samples of sexually mature chickens (G2 and G4) were similar in 1-butanol, 3-methyl- (OL3), 4-heptanol, 2,6-dimethyl-(OL5), 1-octen-3-ol (OL8); 2,3-octanediol (OL15); 1-hexanol, 2-ethyl- (OL20), 1-tetradecanol (OL23) and 2-dodecen-1-ol (OL25), which were more strongly positively correlated to PC1. On the other hand, the drumstick cuts of sexually immature chickens (G1 and G3) showed similar behavior related to hexanol (OL2), 2-heptanol (OL4), 3-octanol (OL9) and 9-decen-1-ol (OL14), which negatively correlated with PC1. In addition, the drumstick meat from immature females (G3) had a higher relationship with the compounds 1-dodecanol (OL24), phenylethyl alcohol (OL16) and 1-hexanol, 3-methyl (OL19), which were positively correlated with PC2.

Similar results were observed for the drumstick meat of sexually immature chickens (G1 and G3) and the drumstick meat of sexually mature females (G4) with respect to the ketone group, in which there was a greater relationship with compound 2-decanone (ONE4) (Fig. 3C). However, the drumstick cuts of sexually mature chickens (G2 and G4) differed from the other groups. The drumstick meat of sexually mature females (G1) showed a relationship with the volatile compounds 2-tridecanone (ONE6) and 3-heptanone, 5-methyl- (ONE3), and these were positively correlated with PC1. The drumstick meat of mature males (G2) showed an interaction with compound 2-heptanone (ONE1), which was positively correlated with PC2.

For the ester group compounds in the drumstick (Fig. 3D), the sexually immature females (G3) differed from the other groups and revealed an interaction with the compounds 2-butenoic acid, ethyl ester, (E)-(ATE1); butanoic acid, 3-methyl-,ethyl ester (ATE3); 2-butenoic acid, 2-methyl-,ethyl ester (ATE5); 2-hexenoic acid, ethyl ester (ATE6) and hexadecanoic acid, ethyl ester (ATE10), which were positively correlated to PC1. The drumstick meat of sexually mature birds (G2 and G4) had a stronger relationship with the compound pentanoic acid, 2-methyl-, anhydride (ATE8), while the drumstick meat of mature males (G2) showed a relationship with the compounds nonyl nitrate (ATE7) and octisalate (ATE9). The meat of immature males (G1) showed no relationship with any of the identified ester compounds.

In Fig. 4A, drumstick cuts from sexually immature chickens (G1 and G3) showed similar behaviors in hexane, 1-methoxy- (ANE1); dodecane (ANE6); tridecane (ANE8); tetradecane (ANE10) and hexadecane (ANE12), and these compounds were positively correlated with PC1. The drumstick meat of male chickens (G1 and G2) was similar in hexane, 1-(methylthio)- (ANE4), which was positively correlated to PC2. In addition, the drumstick meat of sexually mature males showed an interaction with the compound cyclopentane 1-ethyl-2-methyl-(ANE3). The drumstick meat of mature females (G4) did not show a direct relationship with any of the compounds identified in the hydrocarbon group.

The drumstick meat of sexually immature chickens (G1 and G3) was similar regarding the compounds of the terpene group, specifically 1-undecene (ENE9); thiophene, 2-pentyl- (ENE11); 7-tetradecene, (Z)-(ENE14) and 6-tetradecene, (Z)- (ENE15), which correlated positively to PC1. The drumstick meat of males from Groups G1 and G2 was similar in 1-heptene, 5-methyl- (ENE4) and 1,3-hexadiene, 3-ethyl-2-methyl-(ENE7), and these correlated positively to PC2. Additionally, compounds from the terpene group, such as o-oxylene (ENE3), 1-pentene, 2-methyl- (ENE6) and 1-octene, 6-methyl- (ENE12), were found in the drumstick meat of sexually mature males (G2). The drumstick meat of sexually mature females differed from the other groups and was related to the compound cyclohexene, 4-methyl-1-(1-methylethenyl)- (ENE8), which was negatively correlated with PC2 (Fig. 4B).

The drumstick meat of sexually immature males did not show any relation to the compounds identified in the pyrazine, sulfur compound, furan, and carboxylic acid groups (Fig. 4C). The drumstick meat of females (G3 and G4) and sexually mature males differed. The G3 females showed a stronger relationship with the compounds of the negative quadrant of PC1, while females in Group G4 showed a stronger relationship with hexanoic acid (OIC2). Sexually mature males had a stronger relation-

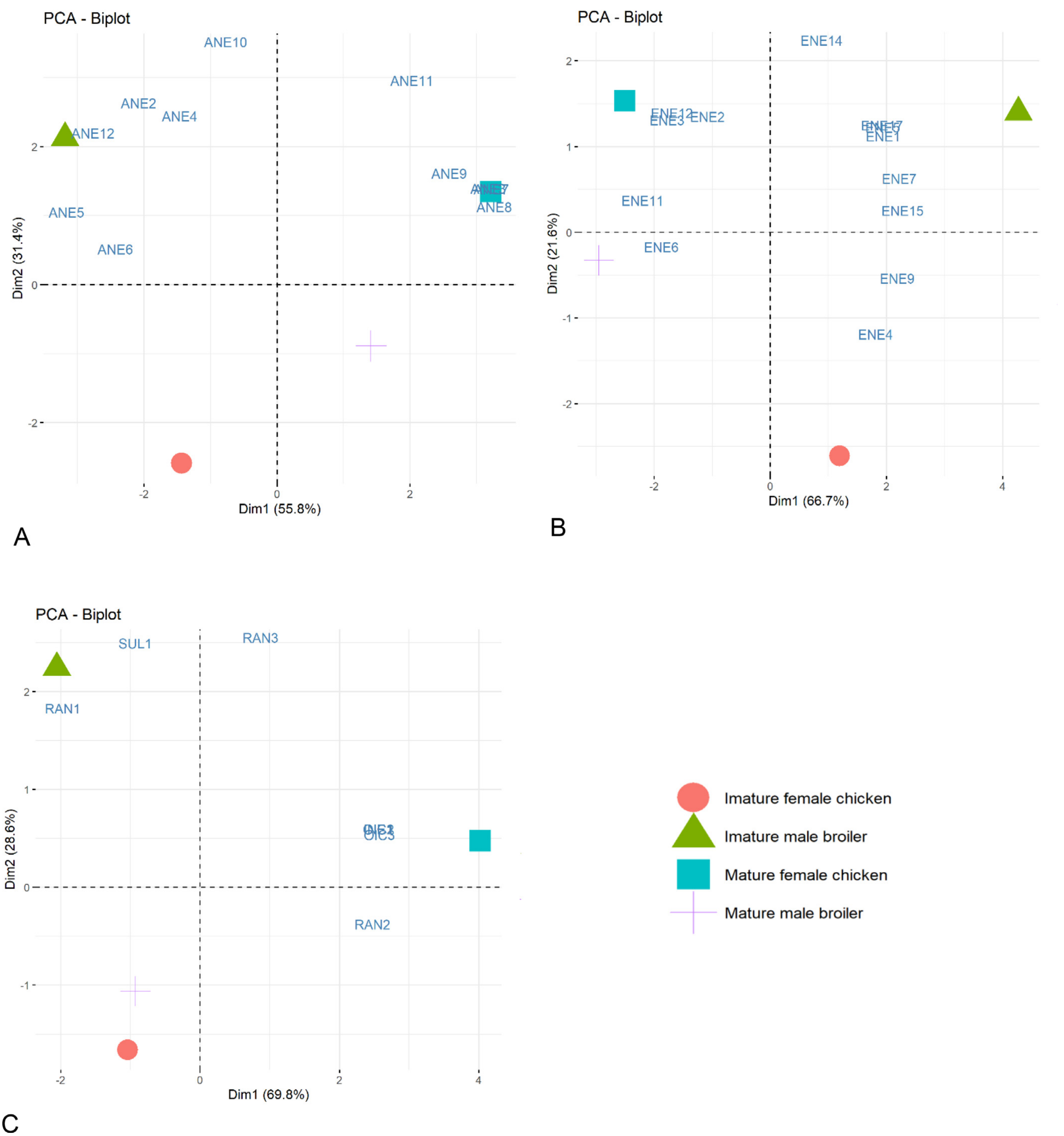

Fig. 2. Principal component analysis of the profile of volatile compounds from the breast cut of chickens raised in a free-range system: (A) hydrocarbons, (B) terpenes, (C) pyrazines, sulfur compounds, furans, and carboxylic acids.
ANE1 – hexane, 1-methoxy; ANE2 – nonane; ANE3 – cyclopentane, 1-ethyl-2-methyl-; ANE4 – hexane, 1-(methylthio)-; ANE5 – undecane; ANE6 – dodecane; ANE7–1-heptyne; ANE8 – tridecane; ANE9 – octadecane; ANE10 – tetradecane; ANE11 – pentadecane; ANE12 – hexadecane. ENE1–1-heptene, 3-methyl-; ENE2–2-octene, (Z); ENE3 – O-oxyline; ENE4–1-heptene, 5-methyl-; ENE5–4-nonene; ENE6–1-pentene, 2-methyl-; ENE7–1,3-hexadiene, 3-ethyl-2-methyl-; ENE8 – cyclohexene, 4-methyl-1-(1-methyletenyl)-; ENE9–1-undecene; ENE10–1,11-dodecadiene; ENE11 – thiophene, 2-pentyl; ENE12–1-octene, 6-methyl-; ENE13–2,10-dodecadiene; ENE14–7-tetradecene, (Z)-; ENE15–6-tetradecene, (Z)-; ENE16–6-hexadecene, (Z)-; ENE17–7-hexadecene, (Z)-; ENE18–2-octene, 4-ethyl-. INE1 – pirazine, 2,6 dimethyl-; SUL1 – trisulfide, dimethyl-; RAN1 – furan, 2-pentyl-; RAN2–2(3H)-furanone, dihydro-5-(2-octenul)-, (Z)-; RAN3–2(3H)-furanone, 5-heptyldihydro-; OIC1 – butanoic acid, 2-methyl-; OIC2 – hexanoic acid; OIC3 – pentanoic acid, 3-ethyl-.

Fig. 3. Principal component analysis of the profile of volatile compounds from the drumstick cuts of chickens raised in a free-range system: (A) aldehydes, (B) alcohols, (C) ketones, and (D) esters

AL1 – hexanal; AL2 – octanal; AL3 – benzene acetaldehyde; AL4–2-octenal, (E)-; AL5 – nonanal; AL6 – dodecanal; AL7–2,5-octadienal, (E,E)-; AL8–2-decenal, (E)-; AL9 – hexanal, 2-ethyl-; AL10–2-butenal, 2-methyl; AL11–2-undecenal, (E)-; AL12 – benzaldehyde, 2-methyl-; AL13 – tetradecanal; AL14 – octadecanal; AL15 – tridecanal; AL16 – pentadecanal; AL17–4-decenal, (Z)-. OL1–2,3-butanediol; OL2 – hexanol; OL3–1-butanol, 3-methyl-; OL4–2-heptanol; OL5–4-heptanol, 2,6-dimethyl-; OL6–2-hepten-1-ol, (E)-; OL7 – heptanol; OL8–1-octen-3-ol; OL9–3-octanol; OL10–1-heptanol, 6-methyl-; OL11–1,3-cyclopenanediol, *cis*; OL12–2-octen-1-ol; OL13–1-octanol; OL14–9-decen-1-ol; OL15–2,3-octanediol; OL16 – phenylethyl alcohol; OL17–4-octanol, 7-methyl-; OL18 – decen-1-decenol; OL19–1-hexanol, 3-methyl-; OL20–1-hexanol, 2-ethyl-; OL21 – hexen-3-ol; OL22–11-dodecenol; OL23–1-tetradecanol; OL24–1-dodecanol; OL25–2-dodecen-1-ol. ONE1–2-heptanone; ONE2–2,5-pyrrolidinedione, 1-chloro-; ONE3–3-heptanone, 5-methyl-; ONE4–2-decanone; ONE5–5,9-undecadien-2-one, 6,10-dimethyl-, (E)-; ONE6–2-tridecanone. ATE1–2-butenoic acid, ethyl ester, (E)-; ATE2 – butanoic acid, 2-methyl-, ethyl ester; ATE3 – butanoic acid, 3-methyl-, ethyl ester; ATE4 – formic acid, hexyl ester; ATE5–2-butenoic acid, 2-methyl-, ethyl ester; ATE6–2-hexenoic acid, ethyl ester; ATE7 – nonyl nitrate; ATE8 – pentanoic acid, 2-methyl-, anhydride; ATE9 – octisalate; ATE10 – hexadecanoic acid, ethyl ester.

Fig. 4. Principal component analysis of the profile of volatile compounds from the drumstick cuts of chickens raised in a free-range system: (A) hydrocarbons, (B) terpenes, (C) sulfur compounds, pyrazines, furans, and carboxylic acids.
ANE1 – hexane, 1-methoxy; ANE2 – nonane; ANE3 – cyclopentane, 1-ethyl-2-methyl-; ANE4 – hexane, 1-(methylthio)-; ANE5 – undecane; ANE6 – dodecane; ANE7–1-heptyne; ANE8 – tridecane; ANE9 – octadecane; ANE10 – tetradecane; ANE11 – pentadecane; ANE12 – hexadecane. ENE1–1-heptene, 3-methyl-; ENE2–2-octene, (Z); ENE3 – O-oxyline; ENE4–1-heptene, 5-methyl-; ENE5–4-nonene; ENE6–1-pentene, 2-methyl-; ENE7–1,3-hexadiene, 3-ethyl-2-methyl-; ENE8 – cyclohexene, 4-methyl-1-(1-methyletenyl)-; ENE9–1-undecene; ENE10–1,11-dodecadiene; ENE11 – thiophene, 2-pentyl; ENE12–1-octene, 6-methyl-; ENE13–2,10-dodecadiene; ENE14–7-tetradecene, (Z)-; ENE15–6-tetradecene, (Z)-; ENE16–6-hexadecene, (Z)-; ENE17–7-hexadecene, (Z)-; ENE18–2-octene, 4-ethyl-. INE1 – pirazine, 2,6 dimethyl-; SUL1 – trisulfide, dimethyl-; RAN1 – furan, 2-pentyl-; RAN2–2(3H)-furanone, dihydro-5-(2-octenul)-, (Z)-; RAN3–2(3H)-furanone, 5-heptyldihydro-; OIC1 – butanoic acid, 2-methyl-; OIC2 – hexanoic acid; OIC3 – pentanoic acid, 3-ethyl-.

Fig. 5. Principal component analysis of the profile of volatile compounds from (A) the breast and (B) drumstick cuts of chickens raised in a free-range system in relation to the identified chemical groups.
AL – aldehyde; OL – alcohol; ANE – hydrocarbons; ATE – ester; ENE – terpene; ONE – ketone; SUL – sulfur compounds; RAN – furans; INE – pyrazines; OIC – carboxylic acids.

ship with the compounds pentanoic acid, 2-ethyl- (OIC3); trisulfide, dimethyl-(SUL1); furan, 2-pentyl-(RAN1) and 2(3H)-furanone, dihydro-5-(2-octenyl)-,(Z)-(RAN2).

Principal component analysis (PCA) of the main chemical groups of volatile compounds identified in this study as a function of sex and sexual maturity explained 86.5% of the variation in breast meat (Fig. 5A) and 86.3% of the variation in drumstick meat (Fig. 5B). The evaluated groups differed in the profile of volatile compounds, as their positions in the PCA graph were dispersed.

The breast meat of sexually immature birds (G1 and G3) was similar with respect to the compounds of the alcohol group (OL), which were negatively correlated with PC1. Furthermore, the breast meat of immature males was related to the sulfur compound (SUL), terpene (ENE), and furan (RAN) groups, which were positively related to PC2, while the breast meat of sexually immature females (G3) differed in the ester (ATE) group in the negative quadrant of PC2. Regarding the breast meat of sexually mature chickens, the meat of females was related to the aldehyde (AL), hydrocarbon (ANE), carboxylic acid (ICO), and pyrazine (INE) groups. The breast meat from mature males was not related to any of the main chemical groups identified in the present study (Fig. 5A).

The drumstick meat of sexually immature males (G1) differed from the G2 and G4 groups in PC1 and was related to the alcohol (OL) and ester (ANE) groups. On the other hand, the drumstick meat of sexually immature females (G3) was similar to that of the G1 group regarding PC1 and PC2, and no relationship with the identified chemical groups was observed (Fig. 5B).

The drumstick meat of sexually mature chickens (G2) differed from the sexually immature groups (G1 and G3) in relation to PC1 and from G4 in relation to PC2. The drumstick meat of sexually mature females showed a relationship with the carboxylic acid group (ICO). On the other hand, mature males had a stronger relationship with the aldehyde (AL), ketone (ONE), terpene (ENE), furan (RAN), and sulfur compound (SUL) groups.

4. Discussion

Among the chemical groups examined in this study, aldehydes, terpenes, and alcohols composed the highest numbers of identified volatile compounds (61.46%). Most aldehydes are formed during the oxidation of fatty acids (Domínguez et al., 2019). In general, aldehydes are the main volatile components of cooked meats; they are very important for creating taste and vary by species as they originate from lipid sources (Domínguez et al., 2019; Mottram, 1998).

Compounds such as hexanal and 2-octenal originate from the oxidation of linoleic and arachidonic fatty acids (Lorenzo, Franco & Carballo, 2014). In this study, sex differences independent of maturity level were observed in relation to these compounds, which could be due to hormonal differences, as the results indicate a greater amount of monounsaturated fatty acids and oleic acid in females (Cruz & Faria, 2019). In addition, meat from chickens raised in a free-range system differs in the lipid profile, with an increase in the amount of polyunsaturated fatty acids (mainly $\omega 3$) due to grazing habits and the consumption of grasses (Castellini, Dal Bosco, Cecilia Mugnai & Pedrazzoli, 2006; Cruz & Faria, 2019). Thus, these differences in lipid composition tend to influence the formation of compounds, such as octanal, which is an aldehyde formed from the autoxidation of oleic acid (Domínguez et al., 2019).

Alcohols also originate mainly from the oxidative decomposition of lipids (Sánches-Peña et al., 2005). Alcohols such as 1-octen-3-ol and 1-octanol are synthesized through the autoxidation of linoleic and oleic fatty acids, respectively (Domínguez et al., 2019; Qi et al., 2017). In addition, alcohols can be formed from aldehydes, such as hexanol, which originates from the reduction in hexanal (Domínguez et al., 2019), and the oxidation of other compounds, such as polyunsaturated fatty acids, which are observed in larger quantities as the age at slaughter increases (Cruz & Faria, 2019).

Hexanal was observed in the breast cut of sexually mature males; this compound was highlighted by Ayseli et al. (2014) as having greater importance and possible influence on the final flavor of the meat. Li et al. (2021) reported higher levels of hexanal among chickens slaughtered at 180 days of age, indicating altered levels of volatile compounds during chicken aging. In larger amounts, this compound is related to an unpleasant rancid flavor; in lower amounts, it is associated with the pleasant aroma of grass (Lorenzo et al., 2014).

In this study, the volatile compounds (aldehydes, alcohols, hydrocarbons, and ketones) in both cuts varied by group. In general, as in the graphic representation, inverse patterns of volatile compounds were observed as a function of sexual maturity independent of sex effect. Thus, the flavor accentuation reported by some authors in birds after reaching sexual maturity (Zanusso & Dionello, 2003) could be related to an increase in the lipid content in birds at older slaughter ages (Souza, Faria & Bressan, 2011) and a greater deposition of polyunsaturated fatty acids (Cruz & Faria, 2019). Moreover, in females, there is a greater accumulation of lipids (Rizzi, Baruchello & Chiericato, 2009), and after sexual maturity, they also undergo a change in their physiological state reflected by an increase in the synthesis of proteins and lipids in the liver (Vignale et al., 2018).

The large number of terpene compounds identified in this study may be related to the foraging habits of free-range birds, as these compounds are formed by the metabolism of plants available in the pasture (Petričević, Radovčić, Lukić, Listeš & Medić, 2018). In this study, the number of terpene compounds in drumstick cuts was increased in male chickens independent of sexual maturity, whereas in the breast, these compounds were increased in sexually immature males and sexually mature females. This could be due to the higher consumption of grasses by males due to increased nutrition requirements (Santos et al., 2005).

Differences in ester compounds were observed as a function of sexual maturity. In both cuts, these compounds were reduced in the meat after the chickens of both sexes reached sexual maturity. Ester compounds are formed by the esterification of medium- and short-chain carboxylic acids with primary and secondary alcohols (Domínguez et al., 2019) and are associated with the formation of aromatic compounds that can influence flavor (Abad, 2018). The lower amounts of esters in sexually immature female birds may be due to the absence of compounds from the carboxylic acid group in these birds, as these compounds participate in the esterification process.

Pyrazines originate from the interaction of sugars – glucose – with sulfur-free amino acids – glycine, alanine, and leucine – during the Maillard reaction (Jayasena et al., 2013; Kosowska et al., 2017). A stronger correlation was observed between the compounds of the pyrazine group and the breast meat of female chickens after reaching sexual maturity, while in the drumstick meat, this correlation was stronger with immature chickens. Therefore, this may suggest a greater number of sugars and amino acids without sulfur in the meat of these birds. Only one sulfur compound was identified in both cuts: trisulfide and dimethyl. Higher proportions of this compound were observed in the breast meat of sexually immature males and in the drumstick meat of sexually mature male chickens. Sulfur and nitrogen compounds, such as cysteine, cystine, and methionine, are synthesized after amino acid bonded with sulfur are denatured by heating (Domínguez et al., 2019). Thus, a greater accumulation of trisulfide and dimethyl in the meat of male broilers may be related to a stronger reaction by this group to cooking.

Another group of compounds associated with the process of lipid oxidation is the ketone group (Domínguez et al., 2019). The compound that showed the greatest expression in both cuts was 3-heptanone, 5-methyl-, with the highest proportion found in chickens after sexual maturity in both sexes. Thus, the higher lipid content in older birds (Li et al., 2021) and greater susceptibility of lipid accumulation due to the increase in the proportion of polyunsaturated fatty acids (Cruz & Faria, 2019) may have led to the higher values of these compounds in sexually mature birds.

In this study, furan compounds were more strongly correlated with the breast cut of mature males and the drumstick cut of sexually immature males. From this group, furan-2-pentyl- occurred in the highest proportions in the breast meat of sexually immature males and in the drumstick meat of mature males. This compound creates a pleasant aroma, with sweet, green, fruity, aromatic, vegetable, and roasted nuances (García-González, Tena, Aparicio-Ruiz & Morales, 2008). In general, these compounds derive from the degradation of thiamine, a vitamin naturally present in meat (Kosowska et al., 2017).

The greater amount of hexanoic acid in both cuts of mature females may be due to the increase in fat content after sexual maturity, as the fat content of females is higher due to the hormonal action on enzymes that participate in lipogenesis (Chen, Hsieh & Chiou, 2006, 2014) in addition to the effect of lipid hydrolysis (triglycerides and phospholipids) (Domínguez et al., 2019).

Therefore, sexual maturity, according to sex, influences volatile compounds. The results showed an increase in the occurrence of volatile compounds in birds that had reached sexual maturity, which may be related to the direct effect of body tissues, in both females and males, where there are differences in the deposit fat and profile of these lipids. During animal aging, compounds related to the intensity of meat flavor increase due to the occurrence of aldehydes and ketones associated with lipid oxidation, a process that is crucial for the development of meat flavor (Li et al., 2021). Mature female chickens have higher levels of total lipids in blood circulation than sexually immature females, mainly when entering laying production (Hawkins & Heald, 1966), which can contribute to an increase in lipid oxidation.

Conclusion

In the meat of chickens raised in a free-range system, 96 volatile compounds belonging to groups of aldehydes, alcohols, terpenes, hydrocarbons, ketones, pyrazines, sulfur compounds, esters, furans, and carboxylic acids were found. The profile of volatile compounds differs according to sex and sexual maturity.

Credit authorship contribution statement

Joanna Oliveira Marçal: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft. Giulia Piva Oliveira and Lidiany Mendonça Zacaroni Lima: Methodology, Formal analysis. Fernando Marcos Rubim, Laryssa Fernandes Correa, Diogo Batista dos Santos and Lais Gabrielle Alvarenga Assis: Methodology. Adriano Geraldo: Supervision, Project administration. Peter Bitencourt Faria: Data curation, Supervision, Project administration, Funding acquisition, Writing – review & editing.

Ethics statements

This study was approved by the Animal Ethics Committee (AEC) of the Federal Institute of Education, Science and Technology of Minas Gerais – Bambuí Campus under number 04/2019.

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.

Acknowledgments

The authors thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG. Process no CVZ – APQ 03268-16). The authors also thank Central of Analysis and Chemical Prospecting of the Federal

University of Lavras, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for supplying a scholarship and Instituto Federal de Educação Ciência e Tecnologia de Minas Gerais (IFMG - Campus Bambuí) and the Vaccinar Nutrição Animal® for providing project resources.

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