Food Science and Engineering
http://ojs.wiserpub.com/index.php/FSE/

UNIVERSAL WISER PUBLISHER

Research Article

Analysis of Volatile Flavor Compounds and Physicochemical Properties in Conventional and Organic Pork Meats Using SPME-GC-MS

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Received: 7 February 2023; **Revised:** 27 April 2023; **Accepted:** 5 May 2023

Abstract: Volatiles responsible for aroma and flavor were investigated in the main types of pork consumed in Spain: duroc and white pigs, the latter reared according to organic and conventional procedures. The main volatiles were detected and identified by SPME-GC-MS technique in three different anatomical parts of the animals: ham, loin and tenderloin. Other physicochemical characteristics such as moisture, pH and tenderness were also evaluated, although no significant correlation was found between them and the volatiles studied. All duroc (minuscule) pork samples presented a higher sum of aldehydes and alcohols (26.8% on average), with pleasant aromas and remarkable values of odorous activity, than the white pork samples (17.5% on average). When comparing the white pig samples, the data showed a higher amount of aldehydes and alcohols in the tenderloin of the organic pork samples (17.9% versus 10.28%), which could benefit the flavor of this anatomical part. However, in the case of ham samples, aldehydes and alcohols were found to be more abundant in the conventional pork samples (22.2% versus 14.0%). The data obtained were subjected to a principal component analysis (PCA) in which a clear association was found between some volatiles and the rearing system adopted. In particular, a relationship was observed between organic pork ham samples and the compound glycerol-1-myristate (pleasant odor). The compound 4-isopropylcyclohexylamine (unpleasant odor) and organic pork loin samples were also correlated. A discriminant analysis (DA) was performed using a selection of volatiles, obtaining valuable results for the distinction of the origin of pork meat, after an adequate validation of the analytical method.

***Keywords*:** duroc, aroma, ecological, pH, moisture, tenderness, multivariate analysis, pork breeds

1. Introduction

Pork is one of the most consumed types of meat in the world and occupies a preference place in the diet of many consumers. It is considered a basic source of beneficial nutrients for humans due to its protein content, as well as its high contribution of vitamins and minerals to the diet. In recent times, the demand for high quality products has increased due to the consumers trend to delve into the search for hygiene, freshness and high organic standards [1].

Meat flavor is the main attribute assessed by the consumer when judging meat quality and is strongly associated with the generation of volatile compounds in the meat product. The "flavor" of a meat is deemed to be the combination of flavors and aromas [2] that confer a specific perception to the palate. Previous studies have revealed the existence

DOI: https://doi.org/10.37256/fse.4220232425

of more than 1,000 volatile compounds in meat, being found aldehydes, ketones, alcohols, acids, esters, ethers, hydrocarbons, heterocycles, sulphur compounds, etc. [3]. But despite the high number of these volatiles, only some of them contribute to aroma, or give rise to other odor-active by-products originated from biochemical reactions that play a key role in the scent and flavor of meat [4].

Macleod et al. [5] demonstrated that most of the characteristic aroma of pork meat are originated through four essential routes: Maillard reaction of amino acids or peptides with reducing sugars, lipid oxidation, reaction between the products obtained in the Maillard reaction with lipid-oxidized products, and degradation of vitamins during the cooking stage.

Given the large number of variables involved in the process of aroma and flavor formation in cooked meat, the study of volatile compounds can be complex and hardly reproducible. However, the number of variables affecting flavor in raw meat is lower [5] and easier to determine, given that the Maillard reaction is not totally developed. Indeed, volatiles analysis in raw meats was demonstrated to provide useful information about aroma precursors [2, 6-7] and also about intrinsic and extrinsic factors related to animals and samples treatments, including feed [8], breeds [9], post mortem treatment, e.g. ageing [10], and genetic variations [11].

The healthy trends of the new society are leading livestock companies to opt for organic and extensive meat production systems, revaluing artisanal procedures and the selection of healthier breeds. Thus, new research into the benefits of this new style of production is flourishing [12-15] proving that the total content of volatile compounds was affected by the production system and were higher in the meat of animals reared extensively.

Also, the balance between the economic factor and the search for more exclusive breeds and breeding makes the duroc meat, which shows high intramuscular fat level, stand out as one of the breeds preferred by consumers in recent times. Several studies have focused on the research of this type of meat, obtaining appreciable similarities with the results obtained for iberian breeds [16].

Nowadays, the high consumption and demand levels for meat products makes it necessary to develop rapid and reliable methods of analysis. Many authors agree that gas chromatography coupled to mass spectrometry with solid-phase microextraction (SPME-GC/MS) is especially useful for the comparison of relative amounts of compounds in different samples, when using the same chemical procedure [17-18].

The objective of this project focuses on investigating and comparing the amount of flavor precursor compounds existing in ham, loin and tenderloin of pork samples from white pigs from organic and conventional breeding, and duroc pigs from conventional breeding. The study of physicochemical parameters (pH, moisture content and tenderness) was carried out, as well as the analysis of volatile compounds by SPME-GC/MS. Also, a statistical analysis of variances (ANOVA) and of principal component analysis (PCA) were used to investigate the relationship between different factors. A discriminant analysis (DA) was performed to explore the distribution and clustering of the samples according to several factors that could be of interest for the improvement of the meat manufacturing process.

2. Materials & methods

2.1 *Sample preparation*

The samples used for this study came from three different types of pigs, which were reared, transported and slaughtered in compliance with the health and animal welfare requirements [19]. The animals were randomly selected from the group of pigs complying with some restrictions: the required weight was 95 ± 10 kg, the sex was the same for the selected animals, and the age was 6 months for the white pigs and 7 months for the duroc pigs.

Samples were kept refrigerated at 3 ± 1 °C for one day, and transported under refrigerated conditions to the laboratory the day after slaughter. Before the analysis, the samples were tempered to room temperature.

Meat from different pigs was used for this project:

- 3 White breed pigs, conventionally reared, females, 6 months old, weighing 89.0 ± 10.4 kg and with a percentage of lean meat of 63.3 ± 2.0%. Cross breed (mother crossbred landrace with large-white and father pietrain with duroc blood paret), fed with fodder enriched with corn, wheat and soybean meal (55% corn, 20% wheat, 20% soybean meal, 1.5% oil, 1% lard, 2.5% amino acids and correctors).

- 3 White breed and organic breeding pigs, females, 6 months old, 88.2 ± 5.5 kg weight and with a percentage of

lean meat of 62.5 ± 1.9%. Cross breed (mother crossbred landrace with large-white and father pietrain with duroc blood paret), fed with fodder enriched with wheat, corn and barley (36% wheat, 22% corn, 35% barley, 5% olein, 2% amino acids and correctors). This pig was reared under the guidelines of Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labeling of organic products.

- 3 Duroc breed pigs, females, 7 months old, weighing 101.4 ± 9.1 kg and with a percentage of lean meat of 54.2 ± 4.7%. 100% pure Duroc breed, fed with fodder enriched with wheat, corn and bran (49% wheat, 25% corn, 12% bran, 5% soybean meal, 2.5% olein, 2% sunflower, 2% beet molasse, 2.5% amino acids and correctors).

Before the pigs slaughtering, a stunning with carbon monoxide was carried out complying with legislation [19]. After slaughter, the butchery was performed and the samples obtained were stored under refrigerated conditions at 4 °C. One day after slaughter, the samples were packaged and transported under refrigerated conditions to the Department of Physical Chemistry Laboratory of the University of Malaga.

The samples transported to the laboratory were from three different muscles from each animal: biceps femoris, in this report referred as "ham", longissimus thoracis et lumborum, referred as "loin", and gluteus medius, referred as "tenderloin". Once the 27 different samples were in the laboratory, a subsampling of three distinct parts of each piece was performed. This path was especially relevant in the case of the pork ham and loin, since, given the size of the initial samples, it was necessary to perform a subsampling in different zones to obtain a representative sample of each piece. 81 samples were finally analyzed to perform this study.

Two sample formats were prepared: minced meat samples and cubic pieces samples of 3 × 3 × 3 cm size. The former ones were used for the analysis of volatiles by SPME-GC/MS and the determination of moisture content, while the latter ones were used for the determination of pH and tenderness.

Between 80-100 g of meat were taken from the aforementioned subsamples for the preparation of the minced meat samples. A grinder (IKA A11, IKA Werke, Staifen, Germany) was used to mince the meat and finally the mixture was homogenized manually.

2.2 *Materials, reagents and methods*

2.2.1 *pH analysis*

A portable pH meter (Sension +, Hach Lange Spain, S.L.U) equipped with a penetration electrode LZW 5053 (Hach Lange Spain, S.L.U) together with a temperature probe (Peaktech 5,110, Peaktech Prüf, Ahrensburg, Germany) was used for pH determination. The equipment was calibrated with calibration standards (Hach Lange Spain, S.L.U.) of 4.01, 7.00 and 9.21 pH values. Before the analysis, the samples were tempered at 25 °C in a water bath (Precisterm, J.P. Selecta S.A., Barcelona, Spain) to minimize the pH differences resulting from the temperature variation between the samples.

2.2.2 *Moisture content*

Moisture content was determined in triplicate for each sample according to ISO1442:1997. For this purpose, ceramic capsules equipped with glass rods and containing between 15 and 20 g of sea sand (Panreac, Panreac Química SLU, Barcelona, Spain) with a grain size between 1 and 2 mm, were dried for 1 hour in an oven (Selecta, J.P. Selecta, Barcelona, Spain) at 102 ± 2 °C. Once tempered in the glass desiccator, provided with silica gel desiccant endowed with humidity indicator (Panreac, Panreac Química SLU, Barcelona, Spain), they were weighed on an analytical balance (Sartorius Entris II Essential Line, Sartorius Lab Instruments GmbH & Co, Goettingen, Germany) annotating the value of their weight to the ten-thousandth figure (M_0).

An approximate mass of 5 g of the crushed samples was added to each porcelain capsule previously dried in the oven, annotating the exact weight of the capsule provided with rod, sand and sample (M_1). 5 ml of 96% v/v ethanol (Panreac, Panreac Química SLU, Barcelona, Spain) was added to each capsule and the rod was used to mix the sample with the sand and ethanol. The capsules were left in a thermal water bath (Precisterm Selecta, J.P. Selecta, Barcelona, Spain) at 80 ± 1 °C for 15 min, before being placed in the oven (Selecta, J.P. Selecta, Barcelona, Spain) at 102 ± 2 °C for 4 hours. Once this time had elapsed, the samples were left to cool in a desiccator and were weighed on an analytical balance, annotating the value of the weight of each capsule after drying (M_2). The difference between M_1 and M_2 was divided by the result of the difference between M_0 and M_1. The resulting value multiplied by 100 gave as a result the

moisture percentage of each sample.

2.2.3 *Determination of toughness and tenderness*

The tenderness is considered the inverse of toughness and is related to the decrease of the shear force value (SFV) [20]. The SFV was measured using a force gauge (mark-10 EG20, Mark-10 Corporation, NY, USA) with a resolution of ± 0.009 kg, equipped with a 4.2 cm long × 2.5 cm wide blade to penetrate the meat. A depth gauge (Physical Test Solutions, Culver City, CA, USA) was used to measure the penetration depth of the blade, with a measuring range up to 5 cm and a resolution of ± 0.01 cm. All samples had the same dimensions, 3 × 3 × 3 cm and were placed under the blade in the same position, so that the cut was made perpendicular to the flesh fibre of the sample. As the blade was penetrating onto the sample, the force gauge indicated the force required to cut the meat and the depth gauge showed the depth to which the blade was located from the surface of the sample. Results were taken for the force required to penetrate each meat sample to a depth equal to 1 cm.

2.2.4 *Determination of volatiles profile*

Several researchers have evaluated fibres and temperature procedures or merged the most suitable ones for the analysis of different analytes in different matrices using SPME-GC/MS. Our aim was to discover the main and most abundant volatiles that could influence the perception of meat aroma, with aldehydes and alcohols being the most commonly studied.

In the literature we found several papers revealing that poly(dimethylsiloxane)-divinylbenzene (PDMS/DVB) fibre could be suitable for our purposes, giving satisfactory results in terms of sensitivity, reproducibility and linearity of response [21], and avoiding the pico-tail effect of highly volatile compounds found by some authors when using carboxene-poly(dimethylsiloxane (CAR/PDMS) fibre [22].

Different temperature procedures were studied by Wang et al. [2], concluding that the optimal SPME conditions were extraction temperature at 80 °C, extraction time of 50 min, and desorption time of 2 min. We took this work as a reference, but found that, in our equipment, the results became satisfactory using a shorter extraction time, which was finally set at 30 min instead of 50 min as recommended in the cited article.

2.00 ± 0.02 g of minced sample were weighed on an analytical balance and mixed in a glass vial with 2 ml of osmosed water (Millipore Elix 3, Millipore Corporation, Massachusetts, USA) and 0.3 ml of 10% NaCl solution prepared in the laboratory from solid NaCl reagent (Panreac, Panreac Química SLU, Barcelona, Spain) and osmosed water. Subsequently, the samples were homogenized in an ultrasonic bath (Selecta 300,514, J.P. Selecta, Barcelona, Spain) for 2 min and measured with SPME-GC/MS, Trace GC gas chromatograph coupled to a mass spectrometer model ITQ 900 with ion trap detector and Autosampler Triplus TSH0 (Thermo Fisher Scientific, Massachusetts, USA).

The experimental conditions were as follows:

- Column: ZB-5 L = 60 m ID = 0.25 mm FT = 0.25 µm (Zebron).
- The warmup ramp was set at 40 °C for 3 min, then 8 °C/min until 250 °C and kept 5 more min at this temperature.
- The sample was adsorbed in a PDMS/DVB fibre of 65 µm and 23 Ga.
- The sample was incubated for 40 min at 80 °C and then extracted for 30 min at the same temperature and under agitation.
- The sample was desorbed in the injector at 250 °C for 2 min.
- The source was kept at 230 °C and the transfer line at 250 °C.
- The mass spectrometer recorded in positive mode m/z = 30-200 in full scan.

The retention times of sample peaks were compared with the internal standards (Sigma-Aldrich, Steinhein, Germany) analyzed under identical conditions (indicated in footnote of Table 1) or, in most cases, identified by comparison in the NIST library database (National Institute of Standards and Technology, Gaithersburg). Compounds were considered correctly identified if the library match factor was 70% or more. The circumstance that the same compound appeared in at least 50% of the samples was also taken into consideration.

The abundance of aromatic compounds was determined by normalising the area of a compound to the total peak area of the chromatogram.

Table 1. Volatile compounds detected by SPME-GC/MS and identified in conventional ham (CH), conventional loin (CL), conventional tenderloin (CT), duroc ham (DH), duroc loin (DL), duroc tenderloin (DT), organic ham (OH), organic loin (OL), organic tenderloin (OT) samples. The retention time of each peak (RT) and the percentage of peak area obtained (% Area ± SD) are summarized. No significant relationship was observed between the volatiles and the anatomical part of meat provenance, ($P \geq 0.05$).

Volatile	RT (min)	CH	CL	CT	DH	DL	DT	OH	OL	OT
		Area (%) ± SD								
3-amino-2-hydroxybenzoic acid[1]	10.72	3.03 ± 0.24	1.37 ± 0.13 ^{qr}	0.39 ± 0.03 ^{x}	2.59 ± 0.27 ^{a}	3.83 ± 0.36 ^{q}	6.94 ± 0.74 ^{x}	3.54 ± 0.42 ^{a}	1.24 ± 0.14 ^{r}	3.94 ± 0.36 ^{x}
1-penten-3-ol[2]	11.48	0.45 ± 0.04 ^{a}	0.54 ± 0.06 ^{q}	0.16 ± 0.01 ^{x}	0.62 ± 0.07 ^{ab}	0.64 ± 0.07 ^{r}	0.15 ± 0.02 ^{y}	0.33 ± 0.04 ^{b}	0.61 ± 0.08 ^{qr}	0.39 ± 0.04 ^{xy}
2-ethoxyethanol	11.77	0.01 ± 0.0006 ^{a}	0.00 ^{q}	0.00 ^{x}	0.00 ^{b}	0.00 ^{r}	0.00 ^{y}	1.96 ± 0.17 ^{ab}	0.89 ± 0.07 ^{qr}	5.25 ± 0.33 ^{xy}
3-metyl-1-butanol[2]	12.46	0.00 ^{a}	0.00 ^{q}	0.00 ^{x}	0.00 ^{b}	0.00 ^{r}	0.00 ^{y}	0.17 ± 0.01 ^{ab}	0.04 ± 0.01 ^{qr}	0.27 ± 0.01 ^{xy}
1-pentanol	13.31	0.14 ± 0.01 ^{a}	0.23 ± 0.03 ^{q}	0.04 ± 0.01 ^{x}	0.24 ± 0.03 ^{ab}	0.23 ± 0.03 ^{r}	0.02 ± 0.0008 ^{y}	0.11 ± 0.02 ^{b}	0.29 ± 0.04 ^{qr}	0.14 ± 0.02 ^{xy}
Hexanal[12]	14.32	8.20 ± 0.98	8.16 ± 1.09	4.71 ± 0.58	9.54 ± 1.57	10.14 ± 1.57	5.15 ± 0.86	7.15 ± 1.28	8.96 ± 1.55	6.66 ± 1.02
3-methylbutanoic acid	14.75	0.00 ^{a}	0.00 ^{q}	0.00	0.00 ^{b}	0.00 ^{r}	0.00	0.02 ± 0.0006 ^{ab}	0.01 ± 0.0004 ^{qr}	0.33 ± 0.04
Hydrocinnamic acid[1]	16.12	2.10 ± 0.32	1.07 ± 0.17 ^{qr}	9.09 ± 1.39 ^{xy}	2.72 ± 0.57	3.17 ± 0.63 ^{q}	5.74 ± 1.22 ^{x}	2.81 ± 0.63	1.09 ± 0.24 ^{r}	4.45 ± 0.88 ^{y}
Heptanal[12]	16.97	1.87 ± 0.22 ^{a}	1.85 ± 0.25 ^{q}	0.71 ± 0.09	3.06 ± 0.50 ^{a}	2.41 ± 0.37 ^{r}	0.73 ± 0.12	0.97 ± 0.17 ^{a}	1.55 ± 0.27 ^{qr}	0.94 ± 0.14
3-octene	18.51	0.46 ± 0.04 ^{a}	0.67 ± 0.07 ^{q}	0.16 ± 0.01 ^{x}	0.93 ± 0.11 ^{a}	0.74 ± 0.08 ^{r}	0.12 ± 0.01 ^{y}	0.18 ± 0.02 ^{a}	0.56 ± 0.07 ^{qr}	0.21 ± 0.02 ^{xy}
Glycerol-1-myristate[1]	18.79	4.14 ± 0.29	3.07 ± 0.26 ^{qr}	2.69 ± 0.20 ^{x}	3.52 ± 0.32 ^{a}	4.43 ± 0.35 ^{q}	2.86 ± 0.26 ^{y}	4.88 ± 0.51 ^{a}	3.64 ± 0.36 ^{r}	4.00 ± 0.31 ^{xy}
2-pentylfuran[2]	19.16	2.17 ± 0.11 ^{a}	2.90 ± 0.18 ^{q}	0.88 ± 0.05 ^{x}	1.49 ± 0.09 ^{ab}	1.65 ± 0.08 ^{q}	0.80 ± 0.05 ^{y}	2.06 ± 0.15 ^{b}	2.61 ± 0.18 ^{q}	1.23 ± 0.06 ^{xy}
4-isopropylcyclohexylamine[1]	19.41	5.10 ± 0.61 ^{a}	4.96 ± 0.66 ^{q}	2.24 ± 0.28	5.64 ± 0.93 ^{b}	5.06 ± 0.78 ^{r}	2.05 ± 0.34	2.79 ± 0.50 ^{ab}	3.90 ± 0.68 ^{qr}	2.16 ± 0.33
Clopidol	19.92	0.36 ± 0.06 ^{a}	0.27 ± 0.05 ^{q}	7.42 ± 1.36 ^{xy}	0.36 ± 0.09 ^{b}	0.56 ± 0.14 ^{r}	1.43 ± 0.37 ^{x}	1.28 ± 0.34 ^{ab}	0.23 ± 0.06 ^{qr}	0.73 ± 0.18 ^{y}
1,3-hexadiene-3-ethyl-2-methyl	20.41	0.58 ± 0.03 ^{a}	0.67 ± 0.04 ^{q}	0.28 ± 0.01 ^{x}	0.84 ± 0.05 ^{ab}	0.85 ± 0.04 ^{q}	0.25 ± 0.02 ^{y}	0.54 ± 0.04 ^{b}	0.79 ± 0.05 ^{q}	2.16 ± 0.10 ^{xy}
E-2-octenal[12]	20.71	0.88 ± 0.07 ^{a}	0.74 ± 0.07 ^{q}	0.52 ± 0.04 ^{x}	1.42 ± 0.15 ^{b}	1.19 ± 0.11 ^{qr}	0.61 ± 0.07 ^{y}	0.83 ± 0.10 ^{ab}	0.81 ± 0.09 ^{r}	0.91 ± 0.08 ^{xy}
1-octanol[12]	20.80	1.95 ± 0.18 ^{a}	2.60 ± 0.27 ^{q}	0.76 ± 0.07 ^{x}	2.74 ± 0.33 ^{a}	2.61 ± 0.29 ^{r}	0.75 ± 0.09 ^{y}	1.13 ± 0.15 ^{a}	2.25 ± 0.29 ^{qr}	1.03 ± 0.11 ^{xy}
1-heptadecyne	21.12	0.00	0.00	8.02 ± 0.51 ^{x}	0.00	0.00	2.43 ± 0.19 ^{x}	0.00	0.00	0.00 ^{x}
1-octadecyne	21.20	0.00 ^{a}	0.00 ^{q}	0.00	3.89 ± 0.35 ^{a}	0.66 ± 0.05 ^{q}	0.00	2.39 ± 0.25 ^{a}	6.39 ± 0.63 ^{q}	0.00
1-hexadecyne	21.25	0.04 ± 0.01 ^{ab}	1.87 ± 0.23 ^{qr}	0.00 ^{x}	0.00 ^{a}	0.00 ^{q}	0.00 ^{y}	0.00 ^{b}	0.00 ^{r}	8.48 ± 1.17 ^{xy}
1-undecyne[1]	21.65	18.51 ± 1.48 ^{ab}	15.38 ± 1.44	10.87 ± 0.91 ^{x}	13.58 ± 1.43 ^{a}	14.41 ± 1.37	10.98 ± 1.17 ^{y}	13.61 ± 1.61 ^{b}	11.70 ± 1.33	8.44 ± 0.78 ^{xy}
Phenylethyl alcohol	22.19	0.00 ^{a}	0.00 ^{qr}	0.00 ^{x}	0.00 ^{b}	0.66 ± 0.15 ^{q}	0.00 ^{y}	0.50 ± 0.13 ^{ab}	0.23 ± 0.06 ^{r}	1.05 ± 0.24 ^{xy}

Table 1. (cont.)

Volatile	RT (min)	CH	CL	CT	DH	DL	DT	OH	OL	OT
		Area (%) ± SD								
Octanoic acid	22.50	0.17 ± 0.01 [a]	0.15 ± 0.01 [q]	0.39 ± 0.02 [x]	0.29 ± 0.02 [a]	0.18 ± 0.01 [q]	0.34 ± 0.03 [y]	0.06 ± 0.01 [b]	0.20 ± 0.02 [q]	0.13 ± 0.01 [xy]
Z-3-hexenal[1]	22.87	0.95 ± 0.12 [a]	0.84 ± 0.12 [q]	0.50 ± 0.07	2.30 ± 0.62 [ab]	1.44 ± 0.24 [qr]	0.56 ± 0.10	0.59 ± 0.11 [b]	0.71 ± 0.13 [r]	0.62 ± 0.10
2-propanol[12]	23.50	1.52 ± 0.14 [a]	0.76 ± 0.08 [q]	0.31 ± 0.03 [x]	1.27 ± 1.45 [b]	0.42 ± 0.05 [qr]	5.05 ± 0.62 [x]	0.68 ± 0.09 [ab]	0.48 ± 0.06 [r]	1.45 ± 0.16 [x]
1-dodecyne	23.72	0.66 ± 0.09 [a]	0.80 ± 0.12 [q]	0.32 ± 0.05	0.33 ± 0.06 [ab]	0.44 ± 0.08 [q]	0.34 ± 0.07	0.57 ± 0.12 [b]	0.62 ± 0.13	0.31 ± 0.06
7,8-dioxabyciclo (4.2.2) dec-9-ene[1]	24.89	3.58 ± 0.32 [a]	2.58 ± 0.27 [q]	2.38 ± 0.22 [x]	5.30 ± 0.64 [a]	4.84 ± 0.53 [qr]	4.49 ± 0.55 [xy]	2.41 ± 0.32 [a]	2.19 ± 0.48 [r]	1.74 ± 0.19 [y]
Histamine[1]	26.01	2.35 ± 0.16 [a]	1.61 ± 0.13 [q]	1.16 ± 0.09 [x]	2.71 ± 0.24 [b]	2.32 ± 0.19 [qr]	0.87 ± 0.08 [y]	1.81 ± 0.19 [ab]	1.69 ± 0.17 [r]	2.01 ± 0.16 z
2-furanacetaldehyde[1]	26.26	1.57 ± 0.09[a]	1.61 ± 0.12 [q]	0.00[x]	1.56 ± 0.12 [b]	1.71 ± 0.11 [r]	0.00 [y]	0.87 ± 0.08 [ab]	1.65 ± 0.14 [qr]	1.47 ± 0.09 [xy]
N-decanoid acid[1]	26.30	0.00	0.00	1.51 ± 0.08 [x]	0.00	0.00	1.90 ± 0.12 [x]	0.00	0.00	0.00 [x]
2-octyn-1-ol[1]	26.75	3.28 ± 0.26 [a]	3.21 ± 0.30 [q]	2.77 ± 0.23 [xy]	5.68 ± 0.60 [a]	3.56 ± 0.34 [r]	1.94 ± 0.21 [x]	1.40 ± 0.17 [a]	2.88 ± 0.33 [qr]	1.79 ± 0.17 [y]
(Z)6,(Z)9-Pentadecadien-1-ol	27.09	0.00	0.00	1.85 ± 0.12 [xy]	0.00	0.00	0.00 [x]	0.34 ± 0.03	0.00	0.00 [y]
1-propanol[12]	27.17	1.96 ± 0.22 [a]	1.43 ± 0.18 [qr]	0.00 [x]	1.06 ± 0.16 [a]	0.24 ± 0.03 [q]	4.24 ± 0.64 [x]	0.35 ± 0.06 [a]	0.12 ± 0.02 [r]	2.98 ± 0.41 [x]
1-tridecyne[1]	27.47	1.84 ± 0.18 [ab]	3.12 ± 0.35 [qr]	0.80 ± 0.08	1.22 ± 0.12 [a]	1.61 ± 0.20 [q]	0.86 ± 0.12	1.24 ± 0.18 [b]	2.89 ± 0.41 [r]	0.81 ± 0.10
Trans-octahydro-1H-indene[1]	27.94	0.00 [ab]	0.00 [q]	2.76 ± 0.45 [x]	0.85 ± 0.19 [a]	0.00 [r]	1.01 ± 0.23 [xy]	0.61 ± 0.15 [b]	0.55 ± 0.13 [qr]	2.52 ± 0.54 [y]
Cyclopentane-1-methyl-3-(2-methylpropyl)	28.48	0.00	0.00 [q]	0.00	0.00	0.00 [r]	0.00	0.00	1.01 ± 0.07 [qr]	0.00
4-pentylbenzaldehyde	28.78	1.47 ± 0.10 [a]	1.76 ± 0.15 [q]	0.48 ± 0.15 [x]	0.57 ± 0.17 [a]	0.74 ± 0.06 [q]	0.76 ± 0.07 [xy]	0.95 ± 0.22 [a]	1.47 ± 0.14 [q]	0.58 ± 0.04 [y]
1-pentadecyne[1]	29.16	2.09 ± 0.19 [a]	3.55 ± 0.37 [q]	1.63 ± 0.14 [x]	1.42 ± 0.13 [a]	1.18 ± 0.13 [qr]	1.15 ± 0.14 [x]	1.68 ± 0.46	3.20 ± 0.41 [q]	1.52 ± 0.16
Tert-butyl methyl ether[1]	30.50	1.71 ± 0.19 [a]	1.31 ± 0.16 [q]	1.27 ± 0.36 [x]	0.84 ± 0.20 [a]	2.46 ± 0.34 [q]	2.57 ± 0.39 [xy]	2.83 ± 0.30 [a]	0.75 ± 0.12 [q]	1.48 ± 0.20 [y]
1,13-tetradecadiene[1]	30.89	4.10 ± 0.41 [ab]	6.39 ± 0.72 [q]	3.48 ± 0.67 [x]	1.49 ± 0.39 [a]	2.10 ± 0.26 [qr]	1.44 ± 0.20 [x]	1.99 ± 0.68 [b]	6.10 ± 0.88 [r]	2.15 ± 0.26 [x]
1-heptadecyne[1]	32.82	5.95 ± 0.95 [ab]	7.64 ± 1.32 [q]	7.06 ± 1.15 [xy]	1.74 ± 0.39 [a]	2.58 ± 0.55 [q]	3.11 ± 0.71 [x]	3.26 ± 0.78 [b]	7.20 ± 1.68	3.83 ± 0.81 [y]

[1]24 volatiles selected as most relevant for this study.
[2]Volatiles identified using standards.
[3]Different superscripts within a row denote statistical differences, according to Tukey´s test ($P \leq 0.05$), between pork origin group (conventional, duroc, organic) within ham (a, b), loin (q, r) and tenderloin (x, y) meat for a compound.

The odor descriptions and threshold values of the volatile compounds were obtained from the MSDS of the products, bibliography [23-24] and the databases http://www.chemicalbook.com and https://pubchem.ncbi.nlm.nih.gov/.

2.2.5 *Statistical analysis*

The effect of physicochemical properties (pH, moisture and tenderness) on aroma (volatiles) was analyzed using the ANOVA procedure of Origin software, version 2018. The significance level was set at 0.05, and Tukey's test was used when the ANOVA found significant differences. The same tool was used to study the relationship between physicochemical properties and the type of breeding or breed of the animal from which the samples were taken. A Spearman's 2-tailed correlation test of significance at a confidence level of 0.05 was performed to assess the correlation between the data obtained for the different physicochemical parameters of meat samples from pigs of different breeds and rearing.

A PCA, using the multivariate calculation application of the same software, was performed to explore and quantify the correlation between flavor precursor volatiles and type of pork samples. A discriminant analysis (DA) was also accomplished to determine the clusters of samples based on the results obtained for six previously selected volatiles.

3. Results

3.1 *Physicochemical properties of samples*

In this experiment, the refrigeration conditions in which the samples were kept and the brief time that elapsed until the analysis was performed, did not provide a propitious environment for the development of microflora that significantly could affect the physicochemical properties of the meat. However, other factors become relevant, such as the biochemical reactions that arise in post-mortem meat, where enzymes, proteins, free amino acids, sugars, vitamins and other substances could intervene in hundreds of chemical reactions, giving rise to products that, most likely, could influence on texture, aroma, tenderness and flavor of the meat [1, 25].

In this study, the variation of moisture content, pH and tenderness of the different samples of conventional, duroc and organic pork was investigated. The analysis was carried out in triplicate, and the average of the three results obtained was represented in the graphs. In Figure 1, the acronyms CH, CL, CT, DH, DL, DT, OH, OL and OT refer to the origin of the sample, being "conventional ham", "conventional loin", "conventional tenderloin", "duroc ham", "duroc loin", "duroc tenderloin", "organic ham", "organic loin" and "organic tenderloin", respectively.

Figure 1a shows the moisture content obtained for the different meat samples, being (73.28 ± 2.64)% the average moisture of all samples, analyzed in triplicate and obtaining a maximum standard deviation (SD) among replicates of 0.13. The averages of moisture content calculated for the different sample types were (74.54 ± 0.21)% for conventional pork samples, (70.86 ± 3.63)% for duroc pork samples and (74.44 ± 1.21)% for organic pork samples. The ANOVA results showed that all the moisture results obtained for different anatomical parts differed significantly related to the breed and rearing ($P \leq 0.05$).

A lower value in the average moisture content was observed in the duroc pork samples, while the same order of magnitude was maintained in the averaged results obtained from conventional and organic white pork samples. This effect was also observed by other authors [26] who noticed that duroc pig breeds, with higher marbling score, presented lower moisture than other breeds, showing analogous results than that obtained in the present work. On the other hand, other researchers have obtained results that corroborate that moisture content and marbling score are not directly related, but there are further parameters that could have a complex influence on the results [9].

The difference in intermuscular and intramuscular marbling fat content (marbling score) between duroc pork samples and other types of samples could explain the lower level of moisture content found in duroc pork samples. To support this hypothesis, the average lean meat percentage was calculated for the samples. The average percentages obtained for duroc pork and white porks reared under conventional and organic procedures were (54.2 ± 3.7)%, (63.3 ± 2.0)% and (62.5 ± 1.9)%, respectively. The results were subjected to ANOVA, using Tukey's test for the evaluation of the means, showing that there is no significant difference between the groups of the organic and conventional white pork meat samples ($P \geq 0.05$), although there is a significant difference between these groups and the duroc pork meat ($P \leq 0.05$). By means of the ANOVA between the moisture score groups and the percentage of lean meat, an association between them was demonstrated ($P \leq 0.05$), which corroborates the relation between the moisture content and the marbling score.

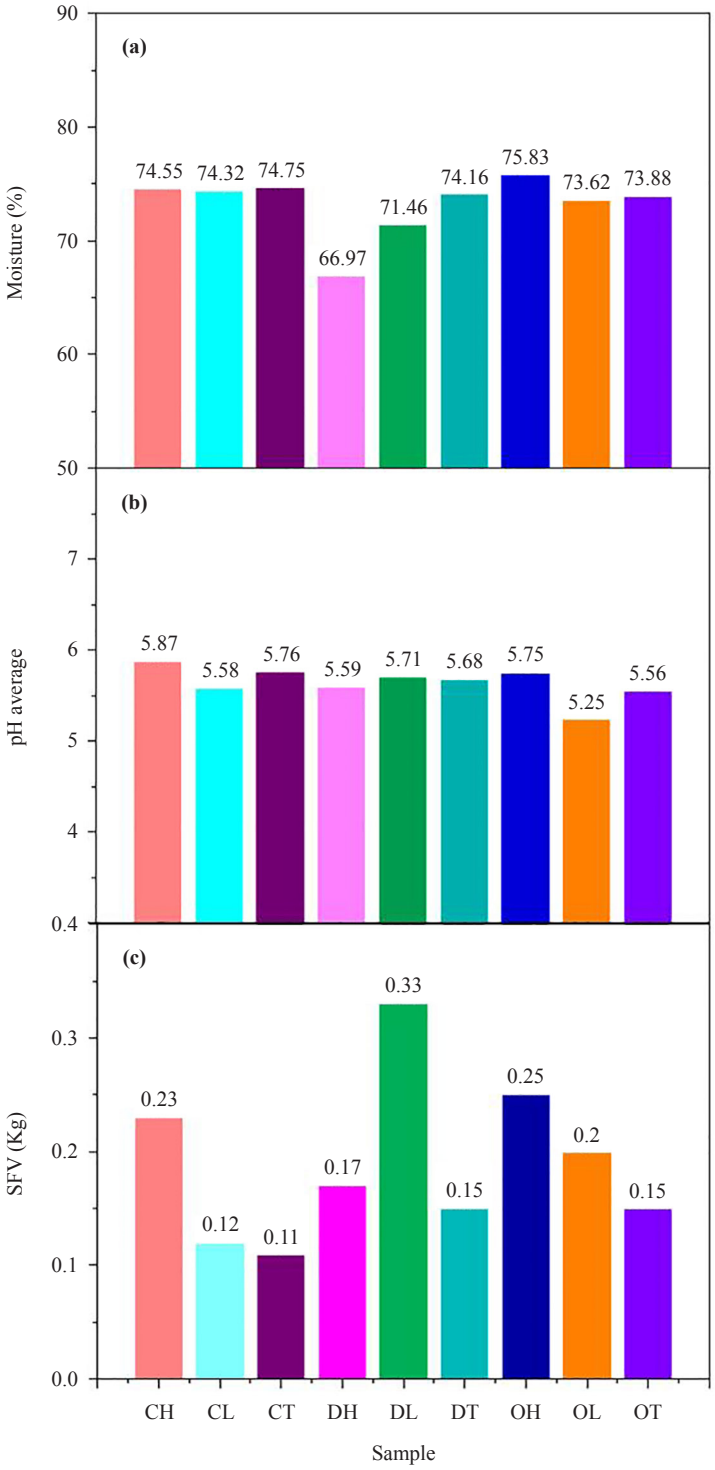

Figure 1. Physicochemical properties related to (a) moisture; (b) pH average and (c) Shear Force Value (SFV) of conventional ham (CH), conventional loin (CL), conventional tenderloin (CT), duroc ham (DH), duroc loin (DL), duroc tenderloin (DT), organic ham (OH), organic loin (OL), organic tenderloin (OT).

The results of pH of samples coming from different animals and anatomies are shown in Figure 1b. The results of the samples from conventional and organic pork showed the same behaviour pattern, with highest pH levels obtained

from ham samples, followed by tenderloin samples and loin samples. Although the pattern is the same, the pH levels obtained in conventional pork samples were higher than those obtained in organic pork samples. However, the trend of pH results obtained in duroc pork samples was different from the others, offering more homogeneous pH values in the different anatomical parts of the animal. The pH averages according to the origin of the sample were 5.74 ± 0.15 for conventional pork samples, 5.66 ± 0.06 for duroc pork samples and 5.52 ± 0.25 for organic pork samples. The result obtained for duroc pork samples agrees with that found by other authors that studied the effect of breed on quality parameters [26-27]. The ANOVA results showed that all the pH results obtained for different anatomical parts differed significantly related to the breed ($P \leq 0.05$).

The tenderness of a piece of meat is affected by many factors, but two of them are dominant: the amount of connective tissue in the meat cut, which usually fluctuates in different anatomical parts from the same animal, and the toughness acquired in the rigor mortis stage, which varies in function of pre- and post-slaughter conditions, and therefore could be controlled, in most cases [28-30]. Figure 1c shows the SFV in kilograms required to perform a 1 cm deep cut on the samples under the conditions indicated in section 2.2.3. The lower the force applied to reach this depth, the greater the tenderness of the meat piece. As can be seen, the tenderloin samples reveal greater tenderness, regardless the type of animal from which the sample was taken. As can be noticed, the behaviour of the result patterns of different anatomical samples from conventional and organic pork are similar again, being higher the tenderness of the tenderloin samples, followed by the loin samples and the ham samples. However, the result patterns from duroc samples behaved different, showing significantly lower tenderness levels in the loin than in the ham. The ANOVA results showed that the tenderness obtained for the different anatomical parts differed significantly ($P \leq 0.05$) in relation to breed and raising, except for the data obtained for the conventional tenderloin samples, which were not statistically different ($P \geq 0.05$) from the duroc or organic pork samples.

In all the muscles studied, it could be observed that conventional pork has a higher level of tenderness than the organic pork, with significant differences detected in the ANOVA. This nuance may be due to the different rearing conditions of both types of animals, gaining the organic pig more space for mobility, which could lead to a more noticeable development of the muscle fiber, and therefore, less tenderness in the meat for consumption. In line with this, studies have shown that conventional pork is often more tender than meat from organic pork production systems, due to lower daily gain in organic production [31], which is known to decrease the proteolytic potential of the muscle at the time of slaughter [32].

A Spearman's 2-tailed correlation test of significance at a confidence level of 0.05 was performed to assess the correlation between the data obtained for the different physicochemical parameters of meat samples from pigs of different breeds and rearing, discovering a correlation significant at 0.05 confidence level for the pH and moisture parameters, showing a p-value of 9.78E-04. This finding could be related to the genetics of the animals [33], the feed received [34-35], the rearing [36], and a variety of other variables that would require further study.

3.2 *Aroma and flavor volatile precursors*

The flavor and aroma formation in meat is related to endogenous enzymatic activities, microbial actions and chemical reaction between natural components [37]. The most important source of aroma compounds is the lipid fraction of meat, that generates acids, aldehydes, ketones and alcohols through the oxidation of phospholipids [38-40]. By-products of fat oxidation are short-chain compounds responsible for the aroma and flavor of meat. In particular, aldehydes, the main aroma contributors studied in this research, represented the secondary oxidation products of mayor meat unsaturated fatty acids, like oleic, linoleic and linolenic [41]. Other aroma compounds found in this study, such as alcohols, are usually the result of free radical-promoted decomposition of saccharides due to lipid oxidation, and hydrocarbons are generated from alkoxy cracking of fatty acids [2].

In this study, 41 volatile compounds were detected and identified in different anatomical parts of conventional, duroc and organic porks, which are summarized in Table 1. In this table, the retention time of each peak (RT) and the percentage of peak area with the resulting standard deviation (% area ± SD) were shown for conventional ham (CH), conventional loin (CL), conventional tenderloin (CT), duroc ham (DH), duroc loin (DL), duroc tenderloin (DT), organic ham (OH), organic loin (OL), organic tenderloin (OT) samples. It should be considered that this study was done with raw meat samples, so the number and quantity of volatiles obtained was significantly lower than that reported by other authors in cooked samples [6, 42-43], where the Maillard reaction and other thermal reactions give rise to important

volatiles responsible for aromas and flavor. However, volatiles with negligible odor activity values (OAV) can be precursors of very potent flavors after the development of thermal reactions, so they were considered in this study.

From the total of 41 volatiles obtained, 24 of them were selected as most relevant and responsible for the volatile flavor of meat (marked in bold in Table 1), basing this selection on the abundance in percentage of peak area, the detection of these volatiles in meat samples performed by other authors using the same instrumental technique [9, 18, 44-46] and the reproducibility of the results obtained for the samples in the analysis.

To determine the effect of the anatomical part of the meat provenance (ham, loin, sirloin) on the volatiles detected, the statistical significance was evaluated by one-way ANOVA. The results obtained showed that no significant interaction between data were found under the confidence level stablished.

Table 2 shows the odor notes, pleasant (positive) or unpleasant (negative) connotation, odor threshold value (OT), provenance of the pork (conventional (C), duroc (D) or organic (O)), and the OAV ± SD for the selected compounds in ham (H), loin (L) and tenderloin (T). The latter parameter allows to obtain a more accurate idea of the aroma-producing capacity of each volatile in a matrix and is defined as the ratio between the concentration of the product and its aroma threshold [7, 47-48]. In this work, a variation of the original OAV calculation method has been performed, replacing concentration by percentage of peak area. Given that the aim is to compare measurements, the OAV calculation method with this modification is acceptable.

Even if the OT value of some volatile compounds were not high enough to generate significant aroma by themselves under the presented conditions, the relevance of any of the volatiles should not be underestimated a priori, since these compounds could be later involved in other reactions (during heat treatment, curing, maturation, etc.), giving rise to other volatiles more aromatically active.

As shown in Table 2, some of the volatile compounds appeared in different proportions in the ham, loin and tenderloin. Hexanal is the most relevant aldehyde compound in the volatile profile since it represents a high percentage of volatiles [49] and has an exceptionally low OT, with an averaged OAV of 30.9%, which indicates that the aroma contribution of this compound to the meat is high. This compound has been commonly found as the major volatile of the aldehyde group in other research published by authors who have explored the existence of volatile compounds in raw, cured and cooked meats [46, 50-51]. Hexanal, arising from the oxidation of linoleic acid [52] as well as from the degradation of unsaturated aldehydes [6, 46], has been reported to provide an aroma of freshly cut grass, fat and fruit to the pork meat, with a relatively low OT value (4.5 ppb). It can be noticed that, in the case of conventional pork, ham and loin samples contained higher amount of hexanal, almost twice the tenderloin samples, resulting similar concentrations in loin and ham samples. Duroc tenderloin meat samples were also favored by an increase in hexanal concentration, which provides a pleasant aroma to these samples.

Heptanal, arising also from the oxidation of linoleic acid and unsaturated fatty acids [6, 45-46], provides the meat with a fruity fatty aroma. This aldehyde showed a higher abundance in duroc pork ham and loin pieces and is considered one of the most active compounds that contributes to the aroma, due to its low odor perception threshold.

The same behaviour was observed when studying the compound Z-3-hexenal, which confers aroma of grass, olives and apple, also with an odor threshold below 1 ppb. Other aroma precursor compounds, with odor thresholds close to 3 ppb, are E-2-octenal and 1-octanol, where in both cases, the highest concentration values were found in the ham and loin pieces of duroc pork. This indicates that lipid oxidation was carried out to a greater extent in the duroc pork ham and loin samples [47].

Alkenes and alkynes were generally observed in higher proportions in the conventional pork samples, as was the case of 1-heptadecyne, 1,13-tetradecadiene and 1-pentadecyne. In the case of 1-undecyne, that was the principal compound of the linear hydrocarbons group, significantly higher values were found in conventional ham pieces. Since it has an aroma note with a neutral connotation and practically absence of odor, the contribution of this volatile to the meat aroma is negligible, as long as no thermal reactions occur.

Just the opposite happens with hydrocinnamic acid, also known as phenyl propanoic acid, being the major compound of the acids group, contributing a sweet aroma of fat, musk, cinnamon and rose. It appears in greater proportion in tenderloin, with much higher values in duroc samples than others.

Some alcohols found in the samples, such as 1-octanol, may be products from the oxidation of unsaturated fatty acids [46], as occurs in the case of some aldehydes, such as heptanal and E-2-octenal. It was also reported that the presence in meat of the latter may come from microbial metabolism [53].

Table 2. Aroma notes, pleasant (+) or unpleasant (-) connotation, odor threshold (OT), and odor activity value (OAV) for ham (H), loin (L) and tenderloin (T) of the selected as relevant compounds in conventional (C), duroc (D) and organic (O) pork samples.

Volatile	Aroma	Connotation	OT (ppb)	Pork	OAV		
					H	L	T
3-amino-2-hydroxybenzoic acid	Urine, irritating	(-)	-	C	-	-	-
				D			
				O			
Hexanal	Fruity. wood. Grassy, citric	(+)	0.28	C	29.29 ± 3.51	29.14 ± 3.89	16.82 ± 2.07
				D	34.07 ± 5.61	36.21 ± 5.61	51.18 ± 3.07
				O	25.54 ± 4.56	32.00 ± 5.55	23.79 ± 3.63
Hidrocinnamic acid	Sweet, cinnamon, vanilla, melon	(+)	-	C	-	-	-
				D			
				O			
Heptanal	Fruity, fatty	(+)	0.18	C	10.39 ± 1.25	10.28 ± 1.37	3.94 ± 0.49
				D	17.00 ± 2.81	13.39 ± 2.08	4.06 ± 0.68
				O	5.39 ± 0.96	8.61 ± 1.49	5.22 ± 0.80
Glycerol-1-myristate	Fatty, coconut, citrus, pineapple	(+)	-	C	-	-	-
				D			
				O			
2-pentylfuran	Fruity, fatty, bean	(+)	6	C	0.36 ± 0.02	0.48 ± 0.03	0.15 ± 0.01
				D	0.25 ± 0.01	0.28 ± 0.01	0.13 ± 0.01
				O	0.34 ± 0.03	0.44 ± 0.03	0.21 ± 0.03
4-isopropylcyclohexylamine	Urine	(-)	-	C	-	-	-
				D			
				O			
E-2-octenal	Rice, nuts, fat	(+)	3	C	0.29 ± 0.02	0.25 ± 0.02	0.17 ± 0.01
				D	0.47 ± 0.05	0.40 ± 0.04	0.2 ± 0.02
				O	0.28 ± 0.03	0.27 ± 0.03	0.30 ± 0.03
1-octanol	Orange, rose, lilium, sweet, fruity	(+)	2.7	C	0.72 ± 0.07	0.96 ± 0.10	0.28 ± 0.03
				D	1.01 ± 0.12	0.97 ± 0.11	0.28 ± 0.03
				O	0.42 ± 0.06	0.83 ± 0.11	0.38 ± 0.04
1-undecyne	Neutral	Neutral	-	C	-	-	-
				D			
				O			
Z-3-hexenal	Grass, olives	(+)	0.25	C	3.80 ± 0.49	3.36 ± 0.48	2.00 ± 0.27
				D	9.20 ± 1.66	5.76 ± 0.98	2.24 ± 0.41
				O	2.36 ± 0.46	2.84 ± 0.54	2.48 ± 0.42
2-propanol	Sweet, fruity	(+)	26000	C	5.81E-05 ± 5.26E-06	2.91E-05 ± 3.02E-06	1.20E-05 ± 1.11E-06
				D	4.90E-05 ± 5.86E-06	1.61E-05 ± 1.78E-06	1.90E-04 ± 2.37E-05
				O	2.61E-05 ± 3.49E-06	1.91E-05 ± 2.37E-06	5.68E-05 ± 6.03E-06

Table 2. (cont.)

Volatile	Aroma	Connotation	OT (ppb)	Pork	OAV		
					H	L	T
7,8-dioxabyciclo (4.2.2) dec-9-ene	Characteristic	(-)	-	C	-	-	-
				D			
				O			
Histamine	Urine	(-)	-	C	-	-	-
				D			
				O			
2-furan acetaldehyde	Sweet, almond, nut, toast, milky notes	(+)	10	C	0.16 ± 0.01	0.16 ± 0.01	0.00
				D	0.16 ± 0.01	0.17 ± 0.01	0. 00
				O	0.09 ± 0.01	0.17 ± 0.01	0.15 ± 0.01
N-decanoid acid	Rancid	(-)	-	C	-	-	-
				D			
				O			
2-octyn-1-ol	Sweet, fruity	(+)	-	C	-	-	-
				D			
				O			
1-propanol	Sweet, fruity	(+)	94	C	2.10E-02 ± 2.29E-03	1.50E-03 ± 1.88E-03	0.00
				D	1.10E-02 ± 1.69E-03	3.00E-03 ± 3.57E-04	4.50E-02 ± 6.86E-03
				O	4.00E-03 ± 6.09E-04	1.00E-03 ± 2.02E-04	3.20E-02 ± 4.36E-03
1-tridecyne	-	Neutral	-	C	-	-	-
				D			
				O			
Trans-hydrindane	-	Neutral	-	C	-	-	-
				D			
				O			
1-pentadecyne	-	Neutral	-	C	-	-	-
				D			
				O			
Tert-butyl methyl ether	Ether, anaesthetic	(-)	-	C	-	-	-
				D			
				O			
1,13-tetradecadiene	-	Neutral	-	C	-	-	-
				D			
				O			
1-heptadecyne	-	Neutral	-	C	-	-	-
				D			
				O			

The compound 2-pentylfuran is a product from n-6 polyunsaturated fatty acids oxidation [54], and provides fruity, fatty and bean odor [55], contributing positive connotation for the meat product.

3.3 *Determination of volatile families*

The 24 volatile compounds selected for the study of meat aroma and flavor were classified into 9 chemical families: (a) cyclic hydrocarbons, (b) linear hydrocarbons, (c) aldehydes, (d) acids, (e) alcohols, (f) esters, (g) ethers, (h) furans and (i) amines.

Figure 2 illustrates the percentage represented by each family of volatiles in the total set of compounds detected in the samples analyzed by SPME-GC/MS. As can be noticed, linear hydrocarbons, providing a negative odor note, represent the highest percentage of volatiles in most of the samples, apart from duroc pork tenderloin samples, where acids, providing a positive odor note, are the predominant compounds. Other authors demonstrated the existence of linear hydrocarbons in raw meat [6], which concentration decreased in cooking process.

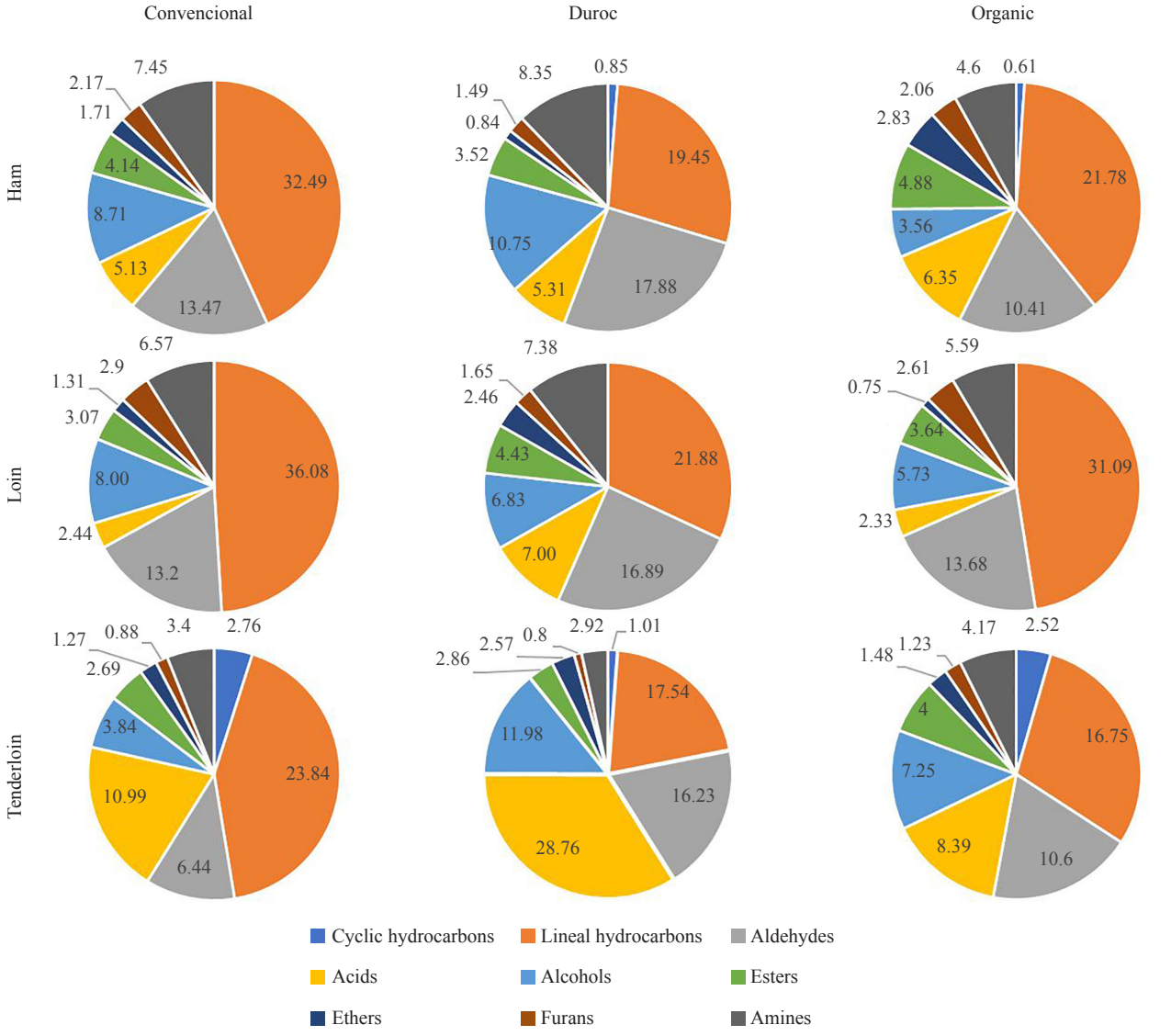

Figure 2. Percentage represented by each family of volatiles (cyclic hydrocarbons, linear hydrocarbons, aldehydes, acids, alcohols, esters, ethers, furans and amines) in the total set of compounds detected in the samples analyzed by SPME-GC/MS in ham, loin and tenderloin of conventional, duroc and organic pork samples.

It can be noticed in loin samples that aldehydes, with pleasant odor note, occupy the second place of abundance. These compounds have been reported as predominant also in cooked and cured meat products, since the treatments give rise to the development of lipid oxidation reactions in these cases [6, 43, 49]. The same trend is observed in conventional, duroc and organic ham pieces, being linear hydrocarbons the predominant compounds, followed by aldehydes.

In this study ketones were not detected, being the most relevant to the contribution of aroma in cooked meats the methylketones. These ketones are the product of beta-keto acids, derived from triglycerides by heat treatment. The absence of ketones could lead to thinking that the leaner cuts of pork studied contain very low-triglyceride levels [6, 46].

Alcohols represent an important percentage of odor-active volatiles, with a pleasant odor note, and are generally detected at higher percentage in duroc pork, except for loin pieces, where the content of alcohols in conventional pork exceeds that found in duroc and organic samples. The abundance of alcohols increases significantly in cooked samples, since they are mainly generated by thermal treatments [49].

Cyclic hydrocarbons, with a negative odor note, are practically absent in ham and loin pieces, but are detected in low concentrations in tenderloin pieces, indicating that the aroma contribution of these compounds is not significant in raw pork. Other authors have already informed that the quantity and types of cyclic hydrocarbons in raw meat is much lower than those found in cooked meat [6].

Furans are generated to a greater extent in the Maillard reaction, so they are generally associated with heat treatment [52]. Despite this, furans, with a positive odor note, are present in this study in all samples, being found in higher concentration level in conventional and organic pork.

In both loin and ham pieces, higher concentration level of amines, with a negative odor note, was detected in duroc pork, followed by conventional pork and organic pork. However, the behaviour pattern adopted in the tenderloin samples is different, with a higher concentration of amines in organic pork, followed by conventional pork and duroc pork.

Acids play a more significant role in raw meat than in cooked meat, although their contribution varies considerably depending on the origin of the meat. Hydrocinnamic acid, mostly detected in tenderloin pieces, contributes with a positive odor note of sweet aroma, vanilla and cinnamon. On the other hand, 3-amino-2-hydroxybenzoic acid, especially abundant in duroc tenderloin samples, provides an aroma with a negative connotation, that could mask the desirable flavor of other volatiles.

Esters are generated by the esterification of some carboxylic acids and alcohols in meat, mainly in raw meat, since it has been revealed that heat treatment leads to a decrease in these compounds [6]. In the samples studied, the ester compounds play a secondary role, but contribute with sweet flavor to the sample, which may be pleasant to the consumer.

The variation in the distribution of compound families in tenderloin pieces is notorious. As can be observed in Figure 2, the abundance of different volatiles is dependent on the origin of pork, being linear hydrocarbons more predominant in conventional and organic pork and being acids the higher representation in the case of duroc pork. For conventional, duroc and organic pork tenderloins, the second place in abundance were for acids, linear hydrocarbons and aldehydes, respectively.

All duroc pork samples presented higher sum of aldehydes and alcohols (26.8% average), with pleasant aromas and remarkable odor activity values, than white pork samples (17.5% average). When the samples of white pork were inter-compared, the data evidenced a higher amount of aldehydes and alcohols in the tenderloin of organic pork samples (17,9% vs. 10.28%), which could benefit the flavor of this anatomical part. However, in the case of the ham samples, aldehydes and alcohols were found to be more abundant in the conventional pork samples (22.2% vs. 14.0%).

3.4 *Identification of main volatiles*

The abundance of the selected volatiles in different pieces of meat from each origin was studied, displaying significant differences between them. This study provides information on the variability of volatile compounds according to the origin of animal. Figure 3 compares the concentration levels of volatiles obtained in ham (a), loin (b) and tenderloin (c) of meat from conventional, duroc and organic pork.

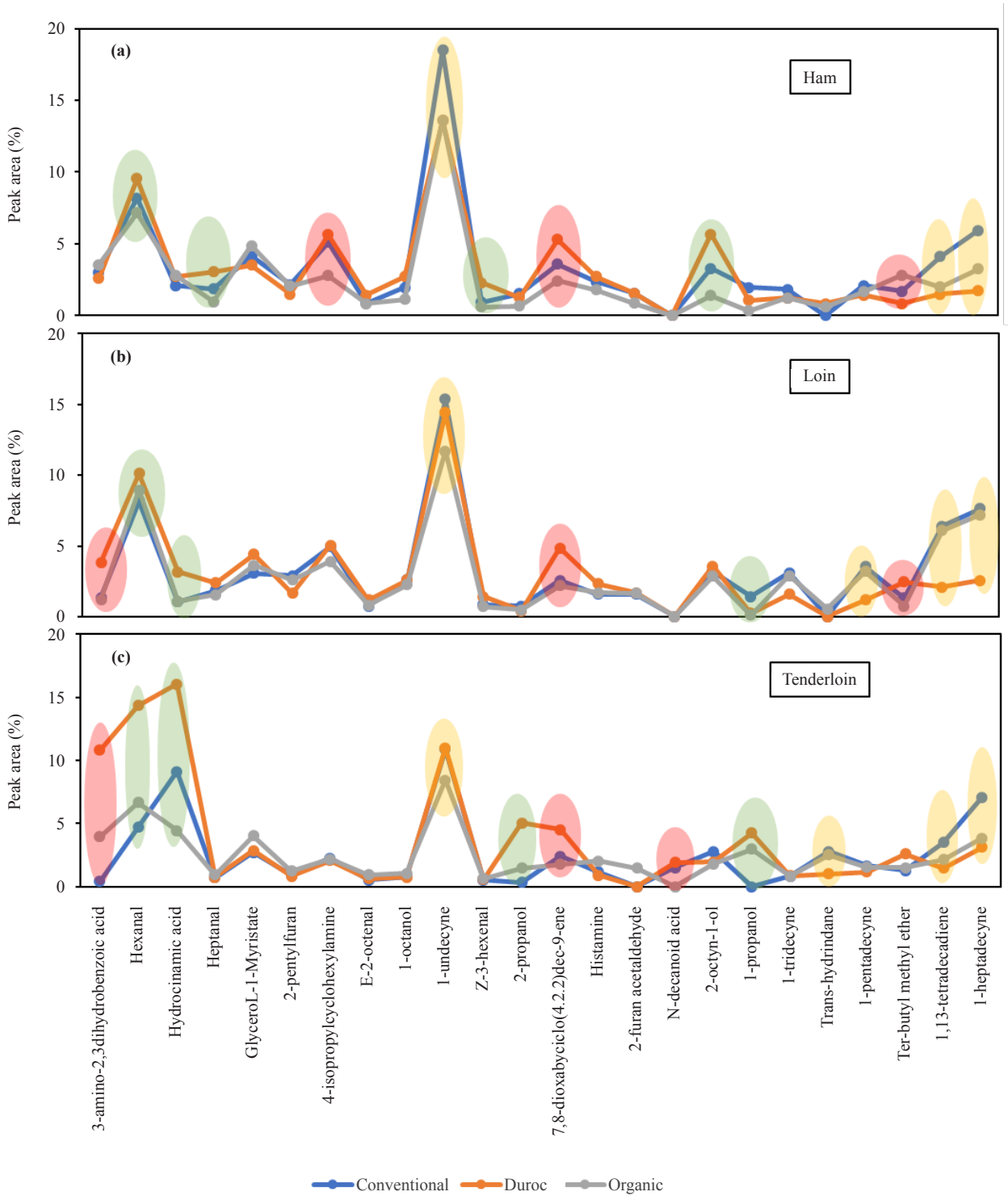

Figure 3. Concentration levels of volatiles obtained in (a) ham, (b) loin and (c) tenderloin of meat from conventional (blue line), duroc (orange line) and organic (grey line) pork samples. Variances greater than 0.5% a^2 in abundance were marked in green, red and yellow according to the connotation of positive, negative or neutral aroma, respectively.

It was established in this assessment that a variance greater than 0.5% a^2 in the abundance results implies a considerable difference in the concentration of volatiles contained in the sample. In Figure 3, the volatile compounds

that present a considerable difference in concentration in samples of conventional pork (blue line), duroc (orange line) and organic (grey line), were marked in green, red and yellow according to the connotation of positive, negative or neutral aroma, respectively.

In this work, it was observed that some volatiles did not show a significant variation in samples from different animals and different pork anatomy, as is the case of glycerol-1-myristate, 2-pentylfuran, E-2-octenal, 1-octanol, histamine, 2-furanacetaldehyde and 1-tridecin, but the remaining compounds showed significant variation in some of the samples.

Figure 3a reveals a difference in some types of aldehydes in ham meat samples, such as hexanal, heptanal and Z-3-hexenal, showing the duroc pork samples the highest concentration levels of these aldehydes. Can also be noticed that the concentration levels of 1-undecine detected in the samples of conventional pork ham are higher than those obtained in the other samples, but this volatile provides an odor note with a neutral connotation. The negative odor perception coming from 4-isopropylcyclohexylamine of conventional and duroc pork is intensified in this group of samples, but also an intensification of 2-octin-1-ol, with positive connotation, is also appreciated in the samples of duroc pork.

In the loin meat samples, Figure 3b, it can be observed how the concentration of some acids is increased in duroc pork, as well as the abundance of tert-butyl methyl ether and 7,8-dioxanbicyclo (4.2.2) dec-9-ene, contributing the two latter with negative connotation in the aroma note. On the other hand, conventional pork meat showed a higher concentration of 1-propanol, which confers a pleasant aroma to these samples.

In the case of the tenderloin meat samples, Figure 3c, it could be perceived that the concentration levels of volatiles were relatively similar in the case of conventional and organic pork samples, however, the duroc pork samples displayed significant differences, showing a greater abundance of compounds with both pleasant and unpleasant aromas. This is the case of compounds such as hydrocinnamic acid, hexanal, 2-propanol and 1-propanol, all with positive aroma note, and 3-amino-2-hydroxybenzoic acid, n-decanoic acid and 7,8-dioxabicyclo (4.2.2) dec-9-ene, with unpleasant aroma. Other neutral volatiles, however, were not more abundant in duroc tenderloin meat than in the tenderloins of conventional and organic samples, except for 1-undecine, whose abundance in duroc tenderloin meat coincided with that of conventional tenderloin. It should be noticed that the compound n-decanoic acid, which has an unpleasant connotation, was only found in samples of tenderloin from conventional and duroc pork, and was not detected in any of the samples of organic pork.

When comparing only the samples of conventional and organic pork tenderloin, it could be noticed that the distribution of the levels of pleasant and unpleasant volatiles benefits in a greater extent the organic pork tenderloin samples, reaching a remarkably similar balance in the case of ham and loin samples.

3.5 *Multivariate statistical analysis*

A multivariate statistical analysis was used to study the effect of volatile compounds in conventional, duroc and organic samples, with the aim of exploring the areas most influenced by the compounds that contribute odor and flavor notes to the samples [45-47].

3.5.1 *Principal components analysis*

A PCA, with a resulting model shown in Figure 4, was performed for the samples of (a) ham, (b) loin and (c) tenderloin. Through this study we can assess the association of volatiles with themselves and with the origins of the samples. In this analysis, only volatiles with positive and negative connotation were considered, being discarded the volatiles with neutral connotation. The abscissa and ordinate axes represent principal component 1 (PC1) and principal component 2 (PC2), respectively, so that the distance of each variable to these axes represents the contribution of that variable to the model. The variables farthest from zero are those that contribute most to the model. For this study, it was established that those compounds that reached an r score $\geq |1, 20|$ in any of the principal components presented a significant influence on the model, being the r score the coordinates PC1 and PC2 of each variable on the model. In the figure, the volatiles that contributed a negative connotation to aroma and flavor were highlighted with red font, in order to visually locate the undesirable areas of the model.

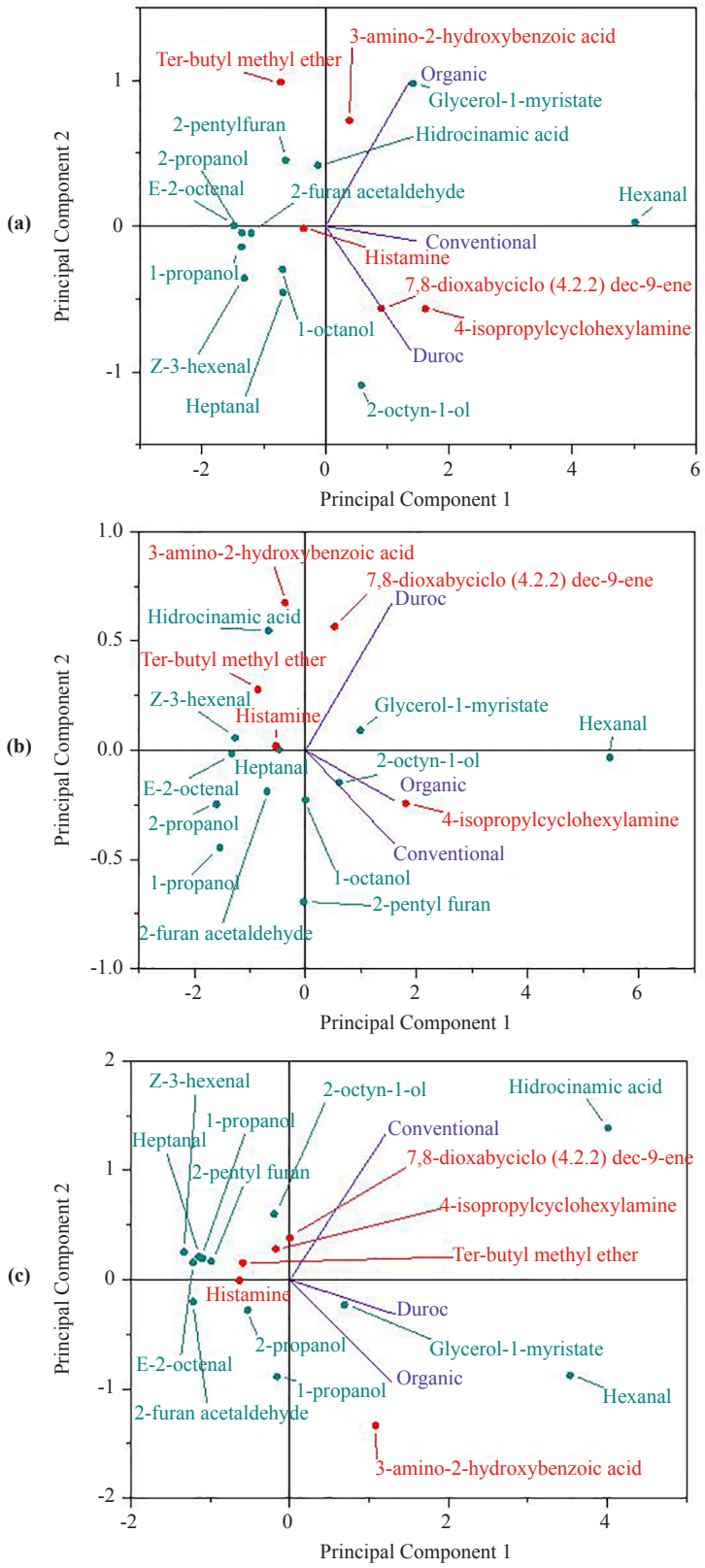

Figure 4. PCA of odor-active compounds of different types of pork meats and performed for (a) ham, (b) loin and (c) tenderloin meat pork samples.

Figure 4a shows the PCA of the ham samples, obtaining a mapping that accumulates 98.53% of the total variance in two principal components, PC1 and PC2, with variance percentages of 87.87% and 10.66%, respectively.

As revealed in figure, there is a large number of volatiles with pleasant connotations grouped in the third quadrant of the graph, but not all of them reached r scores high enough to be considered significant.

Histamine is also located in the third quadrant, with an unpleasant connotation, but with an r score close to zero, making it practically irrelevant in this model. Hexanal, with rPC1 = 5.02, is the compound that contributed most to the model, with other relatively influential compounds also standing out, such as 4-isopropylcyclohexylamine (rPC1 = 1.62), E-2-octenal (rPC1 = -1.48), E-2-octenal (rPC1 = -1.48), glycerol-1-myristate (rPC1 = 1.42), 1-propanol (rPC1 = -1.36), 2-propanol (rPC1 = -1.35), Z-3-hexenal (rPC1 = -1.31) and 2-furanacetaldehyde (rPC1 = -1.20). However, few of these were found to be associated with any type of sample origin.

None of the pork ham samples turned out to be related with the hexanal compound, which was placed in the model in isolation, so a relationship of this compound with any sample type could not be established. On the other hand, the samples of organic pork ham were in the model close to the compound glycerol-1-myristate, with positive connotation, so there was an association of this type of samples with this compound. It can also be seen that the duroc (minuscule) pork ham samples were near to one of the influential compounds in the model, 4-isopropylcyclohexylamine, with a negative connotation, so this compound could be detrimental to the aroma of these samples, although given the distance, it was not possible to stablish a solid association.

Figure 4b presents the PCA of the loin samples, with variance percentages of 94.85% and 4.32% in the first two PC1 and PC2 principal components, respectively, which accumulates 99.17% in total.

In this case was hexanal again the most relevant compound in the model, with rPC1 = 5.48, followed by far by other compounds considered relatively influential (rPC1 or PC2 $\geq$ |1, 20|), such as 4-isopropylcyclohexylamine (rPC1 = 1.80), 2-propanol (rPC1 = -1.61), 1-propanol (rPC1 = -1.54), E-2-octenal (rPC1 = -1.33) and Z-3-hexenal (rPC1 = -1.27). Analysing the Figure 4b the third quadrant was again the zone where the most relevant volatiles were found, but the analyzed samples were far from this zone. As occurred with the ham samples, none of the loin samples was associated with the hexanal compound, being isolated also in this model. However, it was observed that the organic loin samples were located close to the 4-isopropylcyclohexylamine compound, and there was an association between them.

The conventional pork loin samples were more distant to 4-isopropylcyclohexylamine than the organic samples, but, even so, they could be influenced by its unpleasant aroma. The duroc pork loin samples were isolated in the first quadrant, so there was not a clear association of these samples with any of the influential variables.

Figure 4c shows the PCA of the tenderloin samples, representing 95.51% of the total variance in its first two principal components, distributed in 82.53% and 12.99% in PC1 and PC2, respectively.

It can be observed that, in this case, both hexanal (rPC1 = 3.53) and hydrocinnamic acid (rPC1 = 4.01 and rPC2 = 1.39) showed greater influence than the rest of the compounds in this model, both with positive connotation, but they were isolated, meaning that they were not associated with any type of sample origin. Other relevant compounds were 3-amino-2-hydroxybenzoic acid (rPC2 = -1.33), E-2-octenal (rPC1 = -1.22), Z-3-hexenal (rPC1 = -1.33) and 2-furanacetaldehyde (rPC1 = -1.22). Of these compounds, only 3-amino-2-hydroxybenzoic acid conferred an unpleasant aroma and flavor connotation to the samples, being the organic tenderloin samples the closest to this compound, although, given the distance, an association between them could not be assumed.

3.5.2 *Discriminant analysis*

With the aim of making further progress in the statistical analysis, a discriminant analysis (DA) was performed to explore the existing segregation between sample origins according to some volatiles. At the outset, a first selection of volatile compounds was performed, that could be used for making groups of samples. This selection was based on two essential premises:

- Compounds that had been investigated by other authors in similar meat samples and suggested as contributors to the aroma of pork meat.

- Compounds with odor thresholds and aroma identification published on bibliographic references.

Using the volatiles that met these conditions, a first DA screening was performed and were selected the volatiles that presented differentiated zones in the resulting model. The compounds that were selected for DA analysis were hexanal, heptanal, 2-pentylfuran, E-2-octenal, 1-octanol and Z-3-hexenal. These 6 volatile compounds have been

frequently found in previous studies of pork aroma and flavor [45-47] and their physicochemical properties, aroma description and odor threshold data were formerly investigated.

Figure 5 shows the DA of the 6 selected compounds for conventional, duroc and organic pork samples. Three segregated zones are clearly noticed. The first zone, closest to the ordinate axis, includes the compounds E-2-octenal and Z-3-hexenal, followed by a second group of compounds containing heptanal, 2-pentylfuran and 1-octanol, and finally, it was possible to appreciate a third zone where can be found the hexenal compound. The three points identified by the same colour refer to types of pigs used in the study, conventional, duroc and organic. The distribution obtained in this model allowed us to discriminate the type of analyzed samples with a confidence of 66.66%.

To obtain a further rigorous approximation of aroma perception, OAV of the volatile compounds taking part in the DA was calculated.

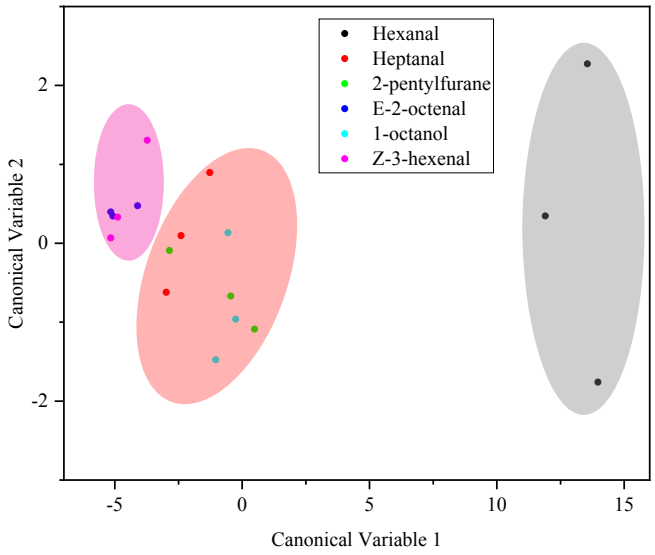

Figure 5. Discrimination analysis (DA) score plot of the 6 selected compounds for conventional, duroc and organic pork samples, where a clear distinction of three clusters of volatiles can be appreciated.

Figure 6 reveals the DA of the OAV results of the 6 selected compounds for the three distinct types of pork samples. This figure shows a more defined discrimination of the clusters of samples, being increased the number of segregated zones to 5. The zone of data with the highest negative magnitude on the abscissa scale corresponded to the results obtained for the 2-pentylfuran and E-2-octenal compounds. The other four zones corresponded to 1-octanol, Z-3-hexenal, heptenal and hexanal, respectively, in order of displacement towards the positive abscissa axis. In the last 4 groups mentioned, labels C, D and O were indicated in the graph, referring to samples from conventional, duroc and organic porks, respectively. The distribution obtained in the DA model using the OAV of 6 cited volatile compounds allowed us to discriminate the origin of an unidentified sample with a confidence of 94.44%.

Given the results obtained in this last DA analysis, a method for the prediction of the origin of the animal from which an unknown sample comes from, by means of a SPME-GC/MS analysis of only 6 volatiles, under the conditions in which this study has been carried out, has been established with a high percentage of confidence. This finding could represent an important advance in the searching for quality assurance in the meat industry and the detection of fraud related to the breed or type of breeding of the animals reared for consumption.

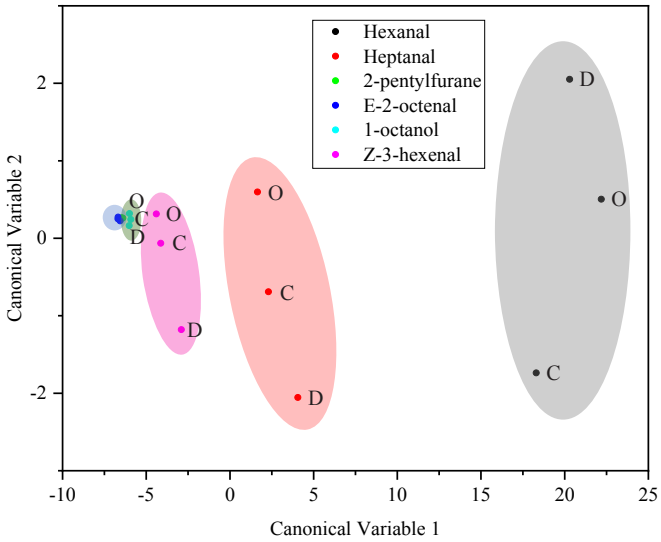

Figure 6. Discrimination analysis (DA) score plot of the odor activity value (OAV) of 6 selected compounds for conventional (C), duroc (D) and organic (O) pork samples, where a clear distinction of five clusters of volatiles can be appreciated.

4. Conclusions

The analysis of physicochemical properties was performed for samples of duroc pork and white pork of conventional and organic rearing systems. The results revealed a lower moisture content for duroc pork samples than for white pork samples. Statistical studies showed that there was a relationship between the moisture and the marbling score presented by the different breeds. In the tenderness study, it was observed that the results obtained for tenderloin from conventionally reared white pigs were significantly higher than those obtained for organic white pigs, attributing this effect to the production system, where the availability of space for the organic pigs rearing system is a distinctive factor. The correlation between physicochemical parameters was studied and a relationship was found between the humidity and pH of the samples according to the origin of pork (duroc pork, organic white pork and conventional white pork) and anatomical part of the samples (ham, loin and tenderloin).

Forty-one compounds of pork meat were detected and identified using SPME-GC-MS, including hydrocarbons, aldehydes, acids, alcohols and esters. The results of volatile quantification showed that duroc pork samples presented higher sum of aldehydes and alcohols, with pleasant aromas and remarkable OAV, than white pork samples. When the samples of white pork were inter-compared, the data evidenced a higher amount of aldehydes and alcohols in the tenderloin of organic pork samples, which could benefit the flavor of this anatomical part. However, in the case of the ham samples, aldehydes and alcohols were found to be more abundant in conventional pork samples.

After performing a statistical analysis of principal components, a relationship between the organic rearing and the compounds glycerol-1-myristate (pleasant), associated with ham samples, and 4-isopropylcyclohexylamine (unpleasant), associated with loin samples was detected. This last compound was also connected to conventional rearing loin samples.

A discriminant analysis of the different sample origins using the OAV of selected compounds resulted in differentiated groups of volatiles. This finding makes it possible to obtain useful information on the origin of any unknown sample by determining the volatiles selected in the DA.

Acknowledgements

The authors would like to thank Dr. Pedro Cañada Rudner, who provided access to the Central Research Support Services of the University of Málaga, and Alianny Brito Pérez, for the technical assistance during the analysis of

samples with SPME-GC-MS. FAMADESA company is also acknowledged for the donation of pork meat. The authors also acknowledge the valuable suggestions from the peer reviewers.

Conflict of interest

The authors declare no competing financial interest.

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