

## Research Article

# Quality Differentiation of *Kraški Pršut*: Exploring the Impact of Weight, Suppliers and Aroma–Sensory Correlations

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In Slovenian, *Kraški pršut*, a dry-cured ham with protected geographical indication (PGI), is traditionally produced. However, the lack of self-sufficiency of local pig producers means the Slovenian pork industry relies on sourcing raw materials (green hams) from foreign countries. This study is aimed at investigating the impact of different weights (16 heavy and 16 light) and suppliers on product quality by analysing 32 samples of dry-cured ham for quality attributes, including colour, texture, chemical composition, descriptive sensory characteristics (such as flavour and texture) and aroma profile. Analyses were conducted on the semimembranosus and biceps femoris muscles, and multivariate statistical methods, including linear discriminant analysis (LDA) and orthogonal partial least squares–discriminant analysis (OPLS-DA), were applied to differentiate between heavy and light hams (based on initial weight categories) and suppliers. Sensory analysis revealed higher softness, juiciness, solubility, marbling and lower saltiness in heavy than in light hams. The SI-heavy hams have received the highest ratings for overall sensory quality. The comparison of volatile organic compound (VOC) profiles indicated the potential for differentiating dry-cured ham by suppliers, particularly within the heavy ham groups, achieving 100% prediction accuracy. Notably, the biceps femoris muscle generally exhibited a higher intensity of positive sensory attributes, such as juiciness, solubility and softness, compared to the semimembranosus, which tended to be firmer and less marbled. The results may inform targeted adjustments in processing techniques—such as refining salting or drying parameters based on ham weight—or guide strategic supplier selection to ensure consistent sensory quality and aroma profiles, thereby enhancing practical applicability.

**Keywords:** descriptive sensory analysis; dry-cured ham; multivariate statistical analysis; volatile compounds

## 1. Introduction

The production of dry-cured ham, traditionally associated with the Mediterranean region [1, 2], has evolved into a global industry, with both consumption and production now widespread [2]. The European Union's quality control systems acknowledge over 30 varieties of dry-cured ham, with approximately half designated as protected designation of origin (PDO) and the remainder as protected geographical indication (PGI) [3]. Certification logos and label information are increasingly important for communicating

characteristics desired by consumers [4, 5]. In Slovenia, the dry-cured ham known as *Kraški pršut* is a traditional product with PGI status [6]. It is highly appreciated by Slovenian consumers and represents an economically significant product at the national level [7]. Furthermore, it serves as a symbol of prestige and is often purchased for special occasions, where price is not a primary selection criterion [8]. Similarly, findings for the Slovenian market suggest that origin is a more important selection attribute for ham than price [5]. Due to the low level of self-sufficiency in pig production in the Karst region, producers of *Kraški pršut* are allowed to

source fresh pork legs (green hams) from within Slovenia and other EU countries [9]. However, the diverse origin of raw materials increases variability in the final product; therefore, the Slovenian meat industry faces significant challenges in maintaining consistent product quality. Additionally, growing consumer demand for leaner products necessitates balancing these preferences with sensory quality of the final product [10].

In general, the distinctive quality and flavour of dry-cured ham are influenced by raw material and processing techniques [10]. Numerous biochemical changes take place during the processing and ripening of dry-cured ham, contributing to sensory characteristics and volatile compounds [11–14]. Flavour, composed of taste and aroma, is a key attribute of dry-cured hams that influences their overall acceptance [15]. It is primarily developed through volatile organic compounds (VOCs), most of which are produced by proteolysis and lipolysis, complex biochemical reactions like lipid oxidation, Maillard reactions and Strecker degradation [16]. The compounds that are responsible for aroma are low molecular weight compounds that are presented in foods at low levels and are highly volatile [17]. These VOCs include aldehydes, carboxylic acids, alcohols, ketones, hydrocarbons and others [18, 19]. Proteolysis activity contributes to the development of texture (e.g., cohesiveness (C) and hardness (H)) [20, 21] and aromatic profile [21], thereby influencing the overall sensory characteristics in the final product [22]. However, excessive proteolysis can severely damage the structures, leading to undesirable textures such as excessive pastiness and softness [20, 23]. One of the most important chemical reactions in fermented meats is the degradation of lipids derived from hydrolysed fatty acids, particularly polyunsaturated fatty acids (PUFAs). This process leads to the formation of hydroperoxides, which are further decomposed into the VOCs such as aldehydes, carboxylic acids, alcohols and esters [24]. Many of these compounds have low odour thresholds and higher volatility, making them major contributors to the flavour of fermented meat products [11, 24, 25]. Additionally, lipids contribute significantly to texture (i.e., mouthfeel), thereby influencing consumer appeal [26].

Over recent decades, several studies have examined the effects of production processes on the physico-chemical, aromatic and sensory traits of various types of dry-cured ham, including Spanish Iberian ([27]); Italian San Daniele, Parma and Toscano ([28]); Croatian Dalmatinski, Drniški, Istarski and Krčki pršut ([29]); and Slovenian *Kraški pršut* [21]. The differentiation between the internal *biceps femoris* (BF) and external *semimembranosus* (SM) muscles has been considered in several studies on various types of dry-cured ham [22, 30–34], including *Kraški pršut* [21, 35]. Different perspectives on the quality of *Kraški pršut* have also been investigated in previous studies [35–37]. Several studies have explored the role of weight-related factors in dry-cured ham production. Toldrá et al. [38] examined the pattern of muscle proteolytic and lipolytic enzymes in light and heavy pigs, though not directly in relation to quality. Similarly, Virgil et al. [39] assessed whether age at slaughter affects the quality of typical Italian heavy dry-cured hams. Recent research

on *Kraški pršut* has examined the effects of ham weight and salting duration on the quality of dry-cured ham produced from heavy pigs [40]. Similarly, Dall'Olio et al. [41] investigated the factors influencing ham weight loss during the initial salting phase in Italian heavy pigs. Furthermore, Malgwi et al. [42] assessed the influence of slaughter weight and sex on growth performance, carcass characteristics and ham traits in Italian heavy pigs. However, there are limited studies investigating the influence of weight and suppliers on the quality of Slovenian dry-cured ham.

Given the increasing demand for high-quality dry-cured ham and the challenges posed by providing the raw material of adequate quality (seasoning aptitude) shortages, it is crucial to establish reliable methods for assessing and ensuring the quality of the final product. This study is aimed at focusing on main objectives: (a) differentiating between ham by weight category (heavy vs. light) and (b) assessing the impact of different suppliers on the final product quality. The results could have significant implications for enhancing the sensory quality of *Kraški pršut*, thereby supporting its market competitiveness and preserving its traditional value. Additionally, the findings are expected to provide valuable insights into the quality of dry-cured ham for the meat industry, enabling producers to optimise production processes, enhance final product consistency and address current challenges related to raw material availability.

## 2. Material and Methods

**2.1. Samples.** Raw materials (green hams) ( $n = 32$ ) were sourced from three different suppliers: (a) K\_light—light hams (imported from Hungary); (b) F\_heavy—heavy hams (imported from Italy); (c) SI\_light—light hams (Slovenia) and (d) SI\_heavy—heavy hams (Slovenia). Each group comprised eight hams, which were categorised based on their weight into light and heavy hams (Table 1). All hams were prepared using the same technological process in accordance with the regulations of the *Kraški Pršut* Consortium. For the production of *Kraški pršut* with a PGI, no specific pig breed is prescribed; fresh hams are selected typically from cross-breeds of noble breeds and must not have been previously frozen [9]. Briefly, the processing consisted of two-stage salting (19 days at 2°C–4°C, without adding nitrates/nitrites), cold resting (88 days at 4°C–6°C and 70%–85% RH) and drying (163 days at 14°C–20°C and 60%–80% RH). Following this, the open ham surface was coated with a protective layer of flour, white pepper and pork fat to prevent excessive desiccation. The hams were then left to mature, reaching a total processing time of 415 days. After maturation, the surface of the hams was trimmed, deboned, hydraulically compressed to the desired shape, vacuum-packed and stored under refrigeration (4°C) [9]. The BF and SM muscles were used for colour determination, texture profile analysis (TPA), chemical analysis, sensory evaluation and volatile profile analysis, as described below.

**2.2. Colour, Chemical and Texture Analysis.** Colour parameters (CIE  $L^*$ ,  $a^*$  and  $b^*$  objective colour parameters) were measured on a freshly cut surface of the BF and SM muscles,

TABLE 1: Properties of *Kraški pršut*.

	K_L	SI_L	SI_H	F_H	Weight SI_L-SI_H	Light SI_L-K_L	Heavy SI_H-F_H	RMSE	p value
Processed ham weight (kg)	8.66 <sup>a</sup>	8.68 <sup>a</sup>	10.30 <sup>b</sup>	10.32 <sup>b</sup>	-1.62 <sup>***</sup>	0.02 <sup>NS</sup>	-0.02 <sup>NS</sup>	0.194	< 0.001
Boneless ham weight (kg)	6.13 <sup>b</sup>	5.82 <sup>a</sup>	7.24 <sup>c</sup>	7.43 <sup>c</sup>	-1.42 <sup>***</sup>	0.31 <sup>*</sup>	-0.19 <sup>NS</sup>	0.292	< 0.001
Boning loss (%)	29.2 <sup>a</sup>	32.9 <sup>b</sup>	29.7 <sup>a</sup>	28.0 <sup>a</sup>	3.2 <sup>‡</sup>	3.6 <sup>*</sup>	1.7 <sup>NS</sup>	3.130	0.028
Subcutaneous fat (mm)	14.3 <sup>a</sup>	13.9 <sup>a</sup>	17.6 <sup>b</sup>	17.6 <sup>b</sup>	-3.7 <sup>***</sup>	-0.4 <sup>NS</sup>	0.0 <sup>NS</sup>	1.951	< 0.001

Abbreviations: SI\_L, SI\_light; SI\_H, SI\_heavy; K\_L, K\_light; F\_H, F\_heavy; RMSE, root mean square error.

<sup>a,b,c</sup>Different superscripts are significantly different ( $p < 0.05$ ).

<sup>NS</sup>  $p > 0.10$ , <sup>‡</sup>  $p < 0.10$ , <sup>\*</sup>  $p < 0.05$ , <sup>\*\*</sup>  $p < 0.01$ , and <sup>\*\*\*</sup>  $p < 0.001$ .

as well as the subcutaneous fat, using a colourimeter (Minolta Chroma Meter CR-300, Osaka, Japan). Each measurement was conducted in triplicate (three measurements across the surface; on three spots across the surface) covering the majority of the muscle cross-section and the average value was used for statistical analysis. Results were expressed as lightness ( $L^*$ ), redness ( $a^*$ , red  $\leftrightarrow$  green) and yellowness ( $b^*$ , yellow  $\leftrightarrow$  blue).

For determination of chemical composition, samples BF and SM dry-cured muscle were sliced into small pieces, cooled in liquid nitrogen and ground to a fine powder using an IKA M20 laboratory mill (IKA-Werke GmbH & Co., Staufen, Germany). Water activity ( $a_w$ ) was measured using an Aqualab 4TE device (Meter Group Inc., Pullman, WA, United States) on 10 g of sample. The lipids, proteins, salt, moisture and proteolysis index (IP) were determined using near-infrared spectroscopy (NIR), measured with the NIR Systems 6500 Monochromator (FOSS NIR Systems, Silver Spring, MD, United States), calibrations developed at the Agricultural Institute of Slovenia.

TPA was performed on the BF and SM muscle using a texture analyser (Ametek Lloyd Instruments, Ltd., Bognor Regis, United Kingdom, fitted with 50-mm diameter compression plate and 500-N load cell), according to Škrlep et al. [43]. Briefly, two slices (15-mm thick) were cut from the main ham sample, and eight samples (20 – mm long  $\times$  20 – mm wide  $\times$  15 – mm high) per muscle were precisely carved out and used for stress relaxation (SR) and TPA. In the TPA test, a repeated 50% compression of the samples was conducted at the compression plate speed of 1 mm/s. The force–time curves were recorded, enabling the calculation of  $H$  (in Newtons), adhesiveness ( $A_3$ ) (in Newton millimetre),  $C$ , chewiness ( $Ch$ ) (in Newtons), gumminess ( $G$ ) (in Newtons) and springiness ( $S$ ) (in millimetre). For the SR test, samples were compressed to 25% of their original height at a speed of 1 mm/s, and the force applied was measured during 90 s of compression, enabling the calculation of the force decay coefficient ( $Y_{90}$ ).

**2.3. Sensory Evaluation.** A quantitative descriptive analysis (QDA) was performed to assess the sensory traits of dry-cured ham [44]. The study with panellists was previously approved by the Committee for Ethical Issues in Professional and Scientific Research of the Agricultural Institute of Slovenia (document EK\_KIS/02/11042022 on 11th April 2023). All panellists agreed to participate in the sensory analysis by signing an informed consent statement. The sen-

sory panel consisted of 10 trained assessors (five female and five male), aged between 28 and 59 years, all of whom were nonsmokers. Preliminary test sessions involved dry-cured hams with a wide range of compositions, flavours and textures to select and validate the sensory descriptors. Then, 16 individual sensory descriptors were established, evaluating the attributes on the entire ham slice (including colour homogeneity, marbling, visible tyrosine crystals, typical matured odour, unpleasant odour and overall sensory quality) or evaluated separately on both BF and SM muscles (including softness, juiciness, solubility, pastiness (excessive softness with mouth coating sensation during mastication), sandiness (perception of crystals during mastication), saltiness, sweetness, bitterness, off-tastes and rancidity). Each descriptor was evaluated using a 9-cm nonstructured scale anchored at both extremes with increasing intensity of sensation. Sensory analysis consisted of four sessions, each with eight samples. Panellists refrained from eating or drinking (except water) for 2 h before the tasting sessions. Two slices from each muscle (1-mm thick, including subcutaneous fat) were placed on a white plastic plate and coded with a randomly chosen three-digit number. Each assessor evaluated each sample individually (in a monadic way, i.e., assessors received the one sample at the time). The samples were randomly distributed across the sensory sessions; each treatment group appeared with two samples within a single sensory session. Water and bread were provided to the panellists to neutralise sensory perceptions between samples.

**2.4. Volatile Compound Analysis by HS-SPME/GC-MS.** The analysis of VOCs was performed according to Babič et al. [45]. Briefly, the extraction was performed using a 50/30  $\mu$ m DVB/CAR/PDMS (2 cm) SPME fibre (Supelco, Bellefonte, United States). Before each extraction, the SPME fibre was conditioned for 5 min at 250°C, followed by a further 20-min conditioning period after analysis. Then, 1 g of homogenised BF and SM muscle was separately weighed into a glass vial (10 mL; Supelco). To each vial, 1 mL of a saturated NaCl solution and 50  $\mu$ L of toluene-d8 (internal standard, 1.2 mg/mL) were added. The vials were capped with a silicone/PTFE septum. The samples were extracted using HS-SPME conditions, with a 60-min equilibration time, 60-min extraction time and 70°C equilibration and extraction temperature. Volatiles were then desorbed for 4 min at 250°C in the GC injector fitted with a straight Ultra Inert SPME Liner (Sigma-Aldrich, Supelco, United States).

The GC-MS analyses were performed using an Agilent 7890B GC and 5977A with mass selective detector (MSD) (Agilent Technologies, United States). Separation was achieved on a VF-WAXms column (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m film thickness, Agilent J&W, United States). Injection was performed in splitless mode with helium as the carrier gas (1.5 mL/min). The temperature programme was as follows: initially, 40°C for 1 min, raised to 150°C at 6°C/min, then to 200°C at 10°C/min and finally to 250°C at 20°C/min and held for 10 min. The total run time was 37 min. The quadrupole, interface and ion source temperatures were 180°C, 280°C and 240°C. Electron ionisation (EI) was performed at 70 eV with an  $m/z$  scan range of 35–300 at a scan rate of 5.2 scans  $s^{-1}$  (full scan mode). Data were acquired using ChemStation software (Agilent, United States). Identification was performed using spectral similarity with the NIST Spectral Database 14 (Agilent, United States) and the retention indices (RIs) where n-alkanes (C9–C23) were used. The confirmation of tentatively identified compounds was made by comparing the calculated RIs according to the equation of Van Den Dool and Kratz [46] with those available in the NIST Chemistry WebBook, SRD 69 and PubChem (normal alkane RI, polar column and custom temperature programme). The relative concentration of each identified compound was calculated by normalising its peak area to the total area of all identified peaks, using toluene-d8 as the internal standard.

**2.5. Statistical Analysis.** The XLSTAT software package (Addinsoft, Long Island, NY, United States, 2019) was used for linear discriminant analysis (LDA). Radar plots were generated using Python (Version 3.13.1.) and Matplotlib library. Significant differences in all analyses were determined using non-parametric Mann–Whitney and Kruskal–Wallis tests ( $p < 0.05$ ). In cases where the Kruskal–Wallis test indicated significant differences ( $p < 0.05$ ), we performed post hoc multiple comparisons using Dunn's test with Bonferroni correction, as implemented in the XLSTAT software. The orthogonal partial least squares–discriminant analysis (OPLS-DA) method was performed (SIMCA 17.0.2, Umetrics, Malmo, Sweden) to differentiate between weight, muscle type and suppliers. Visualisation of group clusters was achieved using LDA and OPLS-DA methods and is based on prior assumptions about sample groupings. LDA, the simplest classifier, requires calculating a centroid for each class and a sufficient sample-to-variable ratio ( $\geq 3$ ), as reported by Marini [47]. As an alternative, OPLS-DA—a modification of PLS-DA—enhances interpretability by separating variance used for predictive purposes from non-predictive variance [48].

The study's performance was assessed using explained variation ( $R^2X$  for PCA and  $R^2Y$  for OPLS-DA) and predictive ability ( $Q^2$ ). The OPLS-DA model's prediction accuracy was measured in terms of sensitivity (true positives) and specificity (true negatives), following the approach by Fiamegos et al. [49]. Additionally, the model's accuracy and  $F1$  score were calculated, where accuracy represents the ratio of correctly labelled samples to the total sample pool, and the  $F1$  score is the harmonic mean of precision

and recall. The  $F1$  score is particularly effective when there is a balance between precision and recall. Discriminant markers were chosen based on their variable importance in projection (VIP) values in the OPLS-DA models, with values above one deemed significant [50, 51].

### 3. Results

**3.1. Differentiation Between Heavy and Light Hams.** In our study, comparative analyses were also conducted between the BF and SM muscles. The results demonstrated that the hams could be distinguished based on colour, texture, chemical composition and aromatic profile with 100% accuracy, whilst sensory analysis achieved 98% accuracy. Given that this aspect has been extensively explored in subsequent studies, it was not the focus of our investigation [22, 30–34], including *Kraški pršut* [21, 35].

The colour, chemical and texture parameters in dry-cured ham are presented in Tables S1, S2 and S3 in Supporting Information. Using the nonparametric Mann–Whitney test ( $p < 0.05$ ), the following parameters were found to be significant for distinguishing between the heavy and light groups:  $b^*$ , Y90,  $H$ ,  $G$ ,  $Ch$ ,  $A3$ ,  $a_w$ , moisture, proteins, salt and IP. An OPLS-DA model was used, resulting in one predictive and one orthogonal component ( $1 + 1$ ), producing an  $R^2X = 0.76$ ,  $R^2Y = 0.62$  and  $Q^2 = 0.58$ . The result (Figure S1) shows that  $a_w$ , salt content,  $H$ ,  $G$  and  $Ch$  with VIP scores  $> 1$  play a pivotal role in differentiating between the groups. The model accuracy was 92%.

The data presented in Figure 1 show that the light group (SI\_light) is predominantly characterised by its salt content,  $G$ ,  $H$  and  $Ch$ . In contrast, the heavy group (SI\_heavy) is distinguished by its  $a_w$ , moisture content and Y90 value. The model achieved an accuracy of 100%. These seven variables are also the most significant parameters based on the Mann–Whitney test.

The results of sensory evaluation are presented in Table S4 in Supporting Information. According to the results, SI\_heavy hams are rated highest in overall sensory quality (Figure 2a) and also achieved the highest ratings in terms of colour homogeneity, marbling and typical matured odour whilst showing the least unpleasant odours. The radar chart further showed differences between SI\_light and SI\_heavy in overall sensory quality, colour homogeneity and marbling. Additionally, distinctions in crystal content were most pronounced within the heavy ham group, with a higher prevalence of tyrosine crystals identified in the F\_heavy group hams. Figure 2b presents sensory descriptors based on the average values of the BF and SM muscles in each of the four groups. In heavy hams, descriptors such as softness, juiciness, solubility and sweetness were more pronounced compared to light hams, whilst saltiness was slightly less pronounced. Figure 2c provides a more detailed comparison of all groups according to SM and BF. The F\_heavy (BF) group was characterised by notably higher values in softness, juiciness, solubility, pastiness and sweetness.

The LDA (Figure S2) results showed that for the heavy ham samples, the more characteristic attributes are sweetness, softness, juiciness and solubility. In contrast, the saltiness was



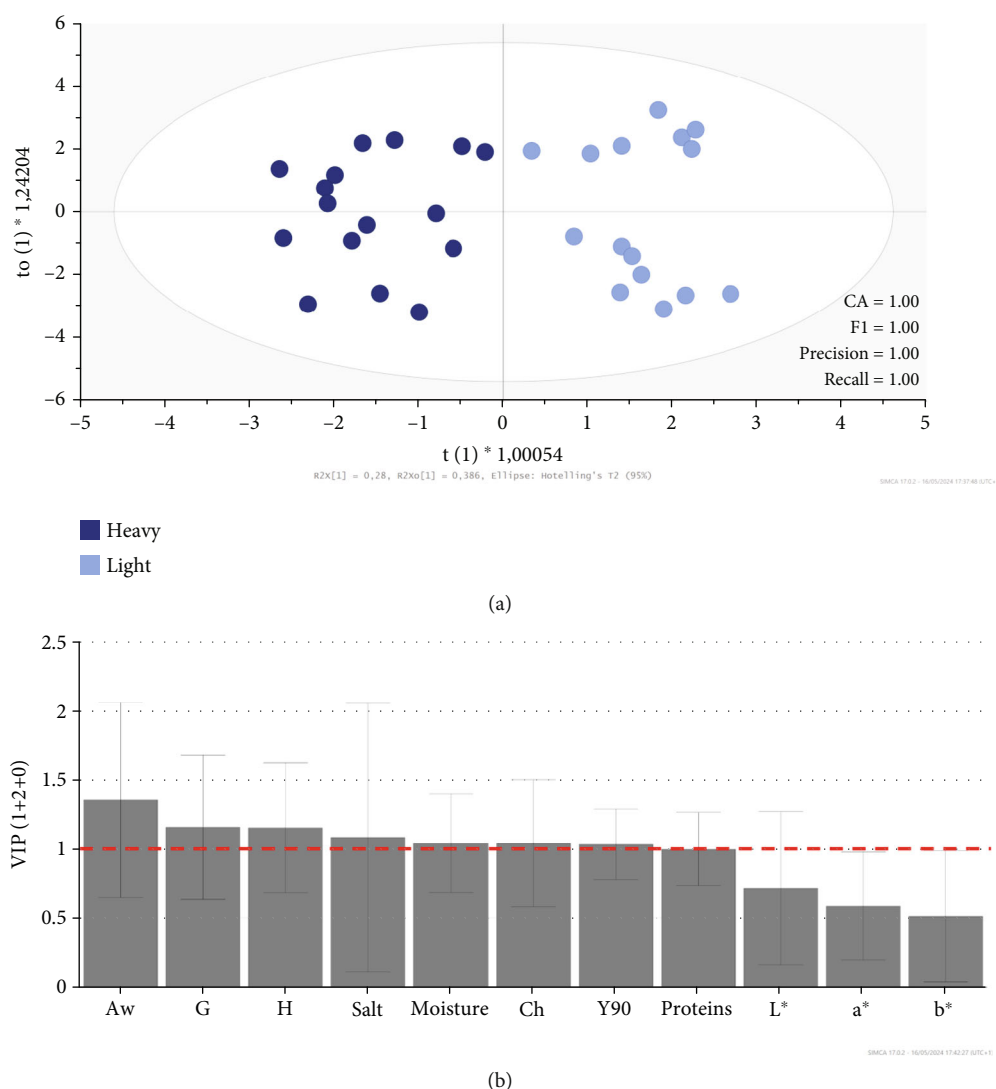


FIGURE 1: OPLS-DA score plots (a) and VIP values (b) in the pairwise comparison of Slovenian heavy and light dry-cured ham groups by colour, texture and chemical analysis. Samples are colour-coded according to their weight class. The ellipse on the score plot denotes the 95% confidence interval. A red dashed line indicates the threshold used to identify the most important variables.

the most prominent characteristic in the light ham samples. Additionally, the OPLS-DA score plots (Figure S3) confirmed that the parameters mentioned above are the most influential for distinguishing between heavy and light hams, achieving a classification accuracy of 91%. The OPLS-DA model was also used to distinguish between Slovenian heavy and light groups based on sensory evaluations. Key findings indicated that softness, juiciness, solubility, sweetness and saltiness with VIP scores  $> 1$  are important for differentiation.

Using HS-SPME/GC-MS, 88 volatile compounds were identified in 32 dry-cured ham samples (Table S5). Any variables with more than 30% missing values were excluded prior to further statistical analysis. Following additional optimisation, this resulted in a final set of 34 compounds, which were classified into several chemical families: aldehydes (28), carboxylic acids (18), alcohols (11), ketones (9), esters (6), furans (4), pyrazines (4), hydrocarbons (4), sulphur-containing compounds (2),

heterocycle (1) and pyridine (1). Distinguishing ham weight based on total volatile compounds is both complex and impractical, highlighting the importance of identifying key volatile compounds through multivariate analysis. For example, by analysing 34 VOCs, it was possible to differentiate between heavy and light groups with an accuracy of 97% (Figure S4). In light hams, key compounds included (E)-oct-2-en-1-ol, hexan-1-ol, pentadecanal, nonanoic acid, oct-1-en-3-ol, 9-octadecanal, hexadecanoic acid and 6-methylhept-5-en-2-one. In contrast, heavy hams were characterised by heptanal, (E)-dec-2-enal, octanal, dodecanoic acid and 9-decanoic acid. These variables were also the most significant parameters based on a Mann-Whitney test. Using the OPLS-DA model (Figure S5), it is also possible to differentiate between Slovenian heavy and light ham groups based on VOCs. The VIP scores for the eight volatile compounds were  $> 1$ , including five acids (dodecanoic acid; (Z)-hexadec-9-enoic acid, 9-decanoic acid,

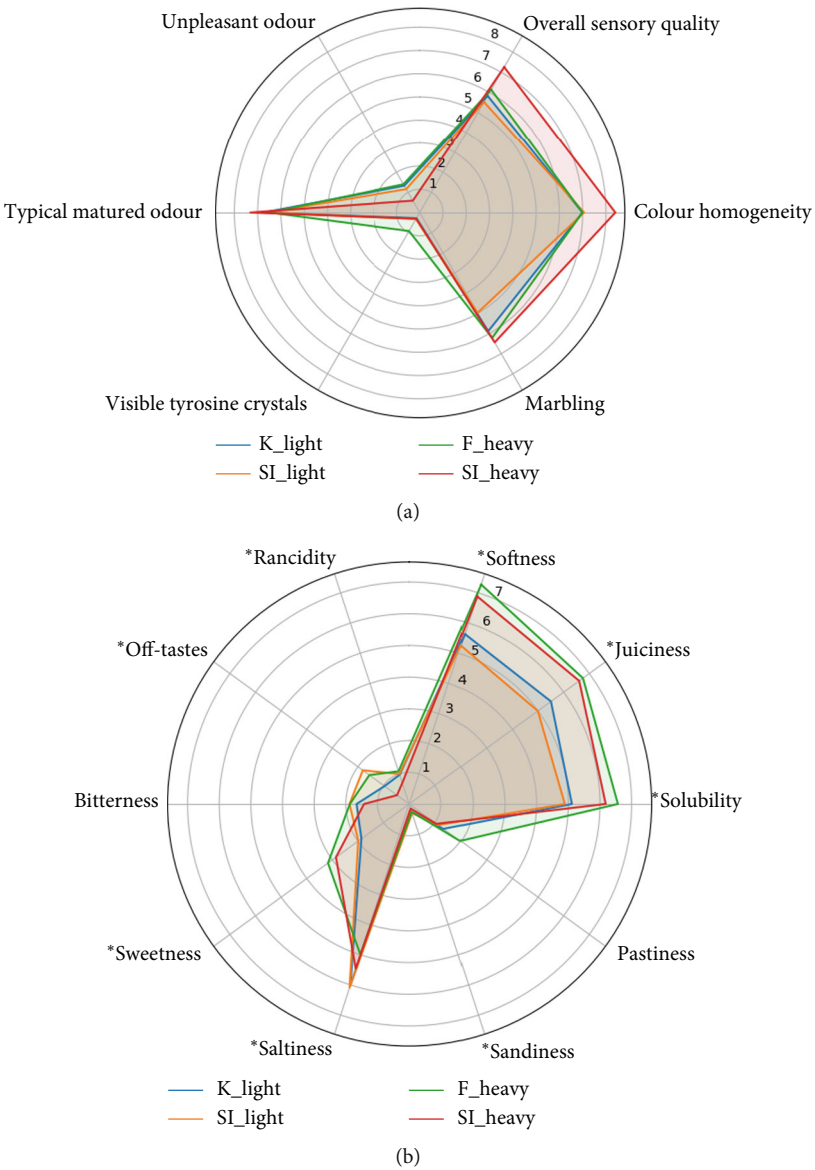


FIGURE 2: Continued.

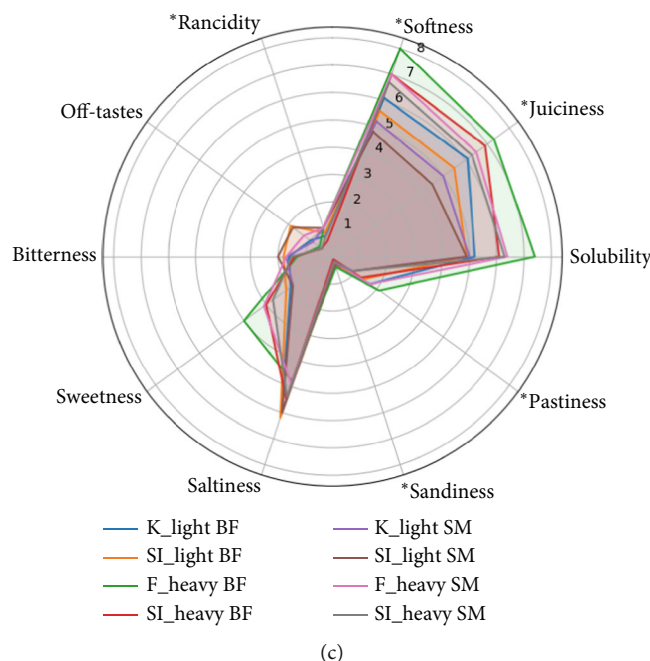


FIGURE 2: A radar chart was based on the score of the sensory attributes of (a) whole slices, (b) average values of the SM and BF muscle across the four groups (highlighting difference between heavy and light groups) and (c) individual SM and BF muscles.

tetradecanoic acid and (9Z,12Z)-octadeca-9,12-dienoic acid), two alcohols (octan-1-ol and 4-methylphenol) and an aldehyde (heptanal). The model achieved a classification accuracy of 94%.

**3.2. Influence of Suppliers.** Since the dataset includes samples from different suppliers, LDA was applied to reveal possible differences based on colour properties, texture profile and chemical composition (Figure S6). The first two discriminant components account for 90.7% of the variance, and four groups are visible in the LDA plots. The most powerful variables for classification were  $a_w$ , salt content, IP, moisture, Y90 and  $b^*$ . The K\_light group showed poorer separation (62.5%). The highest prediction ability (93.8%) was achieved for the F\_heavy group.

The OPLS-DA model, comparing Slovenian and non-Slovenian suppliers (Figure S7), resulted in three predictive and two orthogonal components (3 + 2), producing an  $R^2X = 0.72$ ,  $R^2Y = 0.45$  and  $Q^2 = 0.30$ . According to the results of the Kruskal–Wallis test ( $p < 0.05$ ), the most significant parameters include  $a_w$ , salt, G, H, Ch, moisture and Intramuscular fat (IMF). The OPLS-DA model showed that  $a_w$ , salt, S and G, with VIP scores  $> 1$ , play a pivotal role in differentiating between the groups. The SI\_heavy samples were most similar to F\_heavy, whilst the SI\_light samples were similar to K\_light samples. In the heavy ham group (Figure S7b), there was good separation (100% prediction ability), particularly for the parameters salt,  $a^*$ ,  $a_w$  and S, where the VIP scores were  $> 1$ , indicating these samples were distinct from each other. In contrast, within the light ham group (Figure S7c), the separation, including parameters IMF,  $L^*$ , Y90 and G, where the VIP scores  $> 1$ , was somewhat

poorer (94% prediction ability), suggesting that these hams were more similar to each other.

LDA was applied to reveal possible differences based on sensory evaluation, showing that the distinction between suppliers within the heavy ham group was somewhat more pronounced than within the light ham group (Figure S8a). The first two discriminant components accounted for 93.2% of the variance, with the groups F\_heavy and SI\_heavy being more distinguishable than the SI\_light and K\_light groups in the LDA plots. The strongest descriptors for the heavy group were sweetness, solubility, softness and juiciness. Conversely, the most characteristic parameters for the light group were saltiness, bitterness and sandiness. The K\_light and SI\_light groups showed poor separation (50.0%), with a slightly higher prediction ability for SI\_heavy (56.3%). The highest prediction ability (62.5%) was obtained for the F\_heavy group. The OPLS-DA model was also applied to compare Slovenian and non-Slovenian hams within the heavy and light groups. The analysis revealed that samples within the light ham group were the most similar (accuracy 77%), whereas hams within the heavy group showed greater variability (accuracy 94%).

LDA showed possible differences based on volatile profile, which showed the distinction amongst suppliers across different groups (Figure 3a)—with the F\_heavy samples forming the most distinct group. The OPLS-DA model demonstrated excellent separation (accuracy 100%) amongst samples from the heavy group (Figure 3b) and slightly lower accuracy (94%) for the light group (Figure 3c). The strongest descriptors for the heavy group (SI\_heavy and F\_heavy) were hexadecanal: 6-methylhept-5-en-2-one; (2E,4E)-deca-2,4-dienal; (2E,4E)-nona-2,4-dienal; (E)-non-2-enal; (E)-octadec-9-enal;

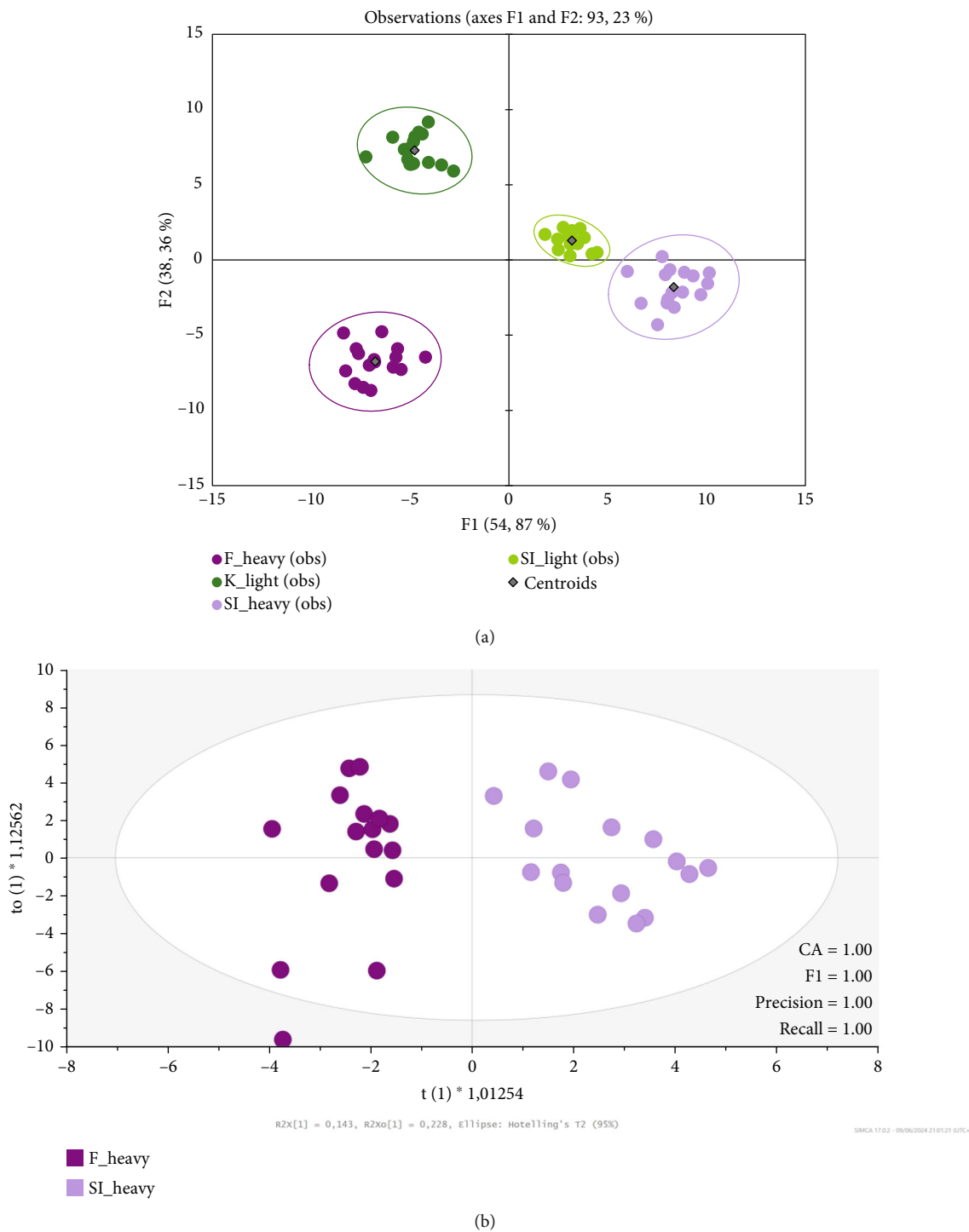


FIGURE 3: Continued.



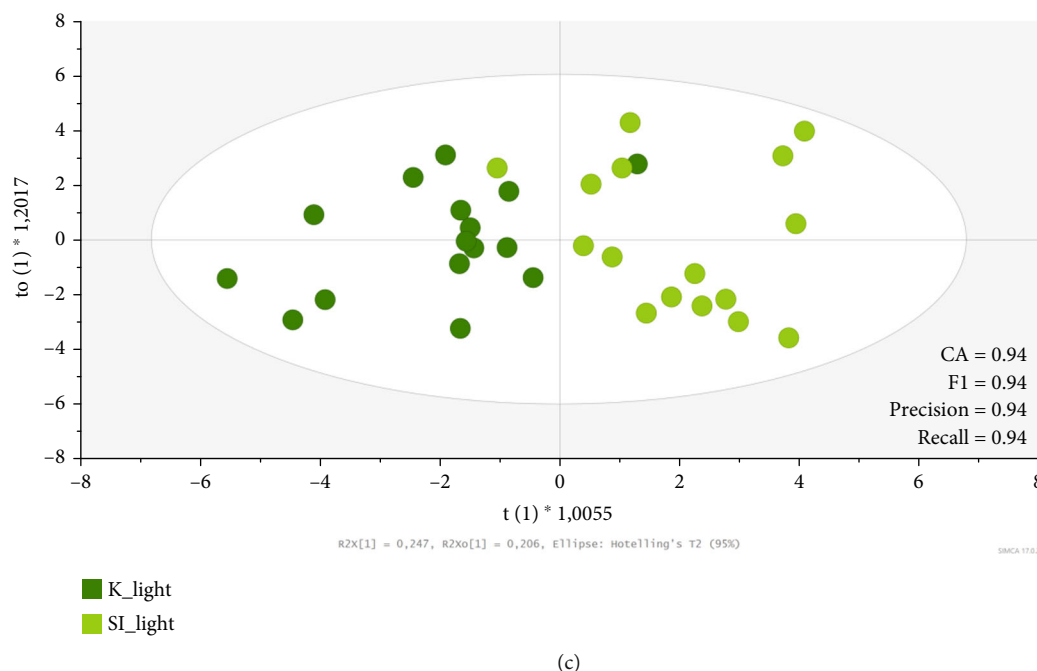


FIGURE 3: (a) Discriminant function score plot illustrates the spatial separation of volatile profiles between different suppliers. OPLS-DA score plots: (b) heavy group—SI\_heavy vs. F\_heavy and (c) light group—SI\_light vs. K\_light. Samples are colour-coded according to their weight class. The ellipse on the score plot denotes the 95% confidence interval. F\_heavy ( $n = 8$ ), K\_light ( $n = 8$ ), SI\_heavy ( $n = 8$ ) and SI\_light ( $n = 8$ ).

(E)-hept-2-enal; (3E)-3-ethyl-2-methylhexa-1,3-diene; (E)-undec-2-enal; hexadecanoic acid and ethyl (E)-hexadec-9-enoate, with VIP scores greater than 1. These compounds play a pivotal role in differentiating between the groups. This second OPLS-DA model, obtained for the comparison between the SI\_heavy and F\_heavy groups, resulted in one predictive and one orthogonal component (1 + 1) producing an  $R^2X = 0.37$ ,  $R^2Y = 0.86$  and  $Q^2 = 0.79$ . The second model, obtained for the comparison between light (SI\_light and K\_light) groups, resulted in one predictive and one orthogonal component (1 + 1), producing an  $R^2X = 0.45$ ,  $R^2Y = 0.63$  and  $Q^2 = 0.45$ . The most significant compounds in this model include bis(2-butoxyethyl) hydrogen phosphate; octadecanoic acid; 5-pentylloxolan-2-one; 5-butyloxolan-2-one; heptanoic acid; dec-9-enoic acid; (E)-undec-2-enal; pentadecenal; (E)-octadec-9-enal; (2E,4E)-deca-2,4-dienal; oct-1-en-3-ol; 3-methylbutanal and (E)-non-2-enal and decanoic acid, with VIP scores  $> 1$ . The comparison of VOC profiles indicated the potential for separating dry-cured ham based on suppliers, especially distinguishing between heavy groups.

#### 4. Discussion

Hams from all four groups were very similar to each other in terms of colour properties. The only statistically significant difference was observed in the lightness ( $L^*$ ) parameter, with the SI\_light group being darker compared to the SI\_heavy group. The decrease in  $L^*$  values is likely attributed to the reduction in water content ( $a_w$ ), as reported by Molinero et al. [13]. Additionally, Fernández-López et al. [52] found

that  $L^*$  values decrease in meat products as salt concentration increases. These findings align with our study, where the hams from both light groups exhibited lower  $a_w$  values and higher salt content compared to both heavy ham groups. No significant ( $p > 0.05$ ) variation in the values of  $a^*$  (redness) and  $b^*$  (yellowness) was found.

In terms of chemical composition, significant differences were observed in all measured parameters except for IMF. The light hams (SI\_light and K\_light) with higher salt concentrations exhibited reduced moisture levels, consequently leading to lower  $a_w$  values. This finding is consistent with previous research [10, 53, 54]. There are also noticeable differences between the groups, with the light hams having a lower  $a_w$  than the heavy hams. Typical  $a_w$  levels in dry-cured meat products range from 0.85 to 0.90 [54], which is consistent with the findings of this study. According to Toldrá et al. [55], proteolytic enzymes remain relatively active at these  $a_w$  values at the end of the production process despite the reduction in the activity of cathepsins and other muscle enzymes, such as aminopeptidases, caused by low  $a_w$ .

The light group (K\_light and SI\_light) showed greater  $H$ ,  $G$  and  $Ch$  texture properties than the heavy group (SI\_heavy and F\_heavy). Moreover, in the heavy ham group, the IP was slightly higher in comparison with light groups, as was softness (positive correlation). The present study agrees with data reported by other authors [21, 56, 57], who observed values between 17.2 and 35.2 in dry-cured hams. Furthermore, salt damages the integrity of muscle fibres, attributing to the A3 and S of meat.

Some authors have reported that the moisture content and protein status of the meat significantly influence changes in the *H* and *Ch* of dry-cured mutton ham [28] and Spanish Cecina de León dried beef meat [13]. Regarding the differentiation between subgroups, the most notable distinctions were observed between the SI\_light and SI\_heavy groups. Dry-cured ham from the SI\_light group showed the hardest texture (*H* 129.3 in SM muscle), whereas ham from the SI\_heavy group was the softest (*H* 61.9 in SM muscle).

The process of proteolysis, which involves the release of peptides and free amino acids, enhances the sensory quality of dry-cured ham by contributing to its distinctive aroma and taste [58]. Across the entire slice, SI\_heavy hams showed the highest rating in overall sensory quality and achieved the greatest assessment for colour homogeneity, marbling and typical matured odour whilst exhibiting the least presence of unpleasant odours. Frank et al. [59] found significantly higher levels of key aroma volatiles in the samples with higher fat content, and the concentration of total branched-chain fatty acids was positively correlated with flavour and overall liking. The similarities between SI\_light and K\_light, as well as between SI\_heavy and F\_heavy groups, are noticeable. Sensory analysis revealed higher softness, juiciness, solubility, marbling and lower saltiness in heavy compared to light hams. The results of the sensory analysis are in line with the results obtained from the instrumental measurement of texture and chemical analysis. Pastiness is quite pronounced in the F\_heavy subgroup, which also has significantly more visible tyrosine crystals, indicating increased proteolysis. Texture defects, including pastiness, softness and crusting, often impede effective slicing and produce an undesirable mouth-coating sensation, highlighting the critical role of texture in determining both distributors and consumer acceptability. Pastiness, in particular, represents a textural defect observed in dry-cured ham, arising from the excessive degradation of muscle protein structures. This defect is attributed to the activity of endogenous enzymes and is closely associated with excessive proteolysis [60]. Juiciness had a positive correlation with moisture content Molinero et al. [13], which was also more pronounced in the group of heavy hams.

The sensory evaluation of ham is closely linked to the composition of VOCs. These VOCs have distinct characteristics and influence each other, contributing to the aroma diversity of hams. This pattern aligns with the volatile profiles observed in similar studies on dry-cured meat products [11, 21, 29, 54].

Aldehydes are the most abundant volatile compounds in *Kraški pršut*, contributing significantly to their unique flavour due to their rapid formation during lipid oxidation and low thresholds [61]. These compounds contribute sweet, floral, grassy and fruity notes to ham [11, 62]. Those differentiating aldehydes based on weight include heptanal, (E)-dec-2-enal, octanal, pentadecanal, 9-octadecanal, tetradecanal, pentylbenzaldehyde, heptadecanal and (2E,4E)-nona-2,4-dienal. Heptanal and octanal, secondary products of (Z)-octadec-9-enoic acid oxidation [63–65] contribute ham-like [66–68], green/grassy [66, 69] and in the case of heptanal, also fruity, citrus, herbal and fatty notes [66, 68, 69], and besides *Kraški pršut* [21] are found in other hams,

including, for example, Parma [65], Toscano [70, 71], Iberian [12, 72] and Jamón Serrano [60]. (E)-dec-2-enal, related to the autoxidation of unsaturated fatty acids [65], is present in Parma [65], Iberian [72], Croatian [29] and Xinjiang dry-cured mutton ham [11]. Although pentadecanal, with its low volatility, does not directly affect the aroma [65], it is prevalent in Mediterranean hams like Parma, Toscano, Iberian and Istrian [15, 29, 65, 68, 71]. Significant descriptors, such as 9-octadecanal, formed from fatty acid oxidation [71], are present in Toscano [70, 71], Croatian [29] and Parma ham [65]. Tetradecanal appears in *Kraški pršut* [21], Toscano [70, 71], Croatian [29], Istrian [15], Iberian [68], Xinjiang dry-cured mutton ham [11] and Cinta Senese dry-cured ham [73]. Aldehydes with low odour thresholds, such as 2-methylbutanal, 3-methylbutanal, pentanal, hexanal, benzaldehyde and 2-phenylacetaldehyde, are crucial in defining “cured-ham” or “aged” aroma, whilst oxidation products like nonanal and 2-hexanal contribute to rancid notes [30].

Acids most potentially originate from lipid oxidation [19]. High molecular weight acids do not directly influence the flavour of the ham due to their high perception threshold [34, 65, 70]. These acids may act as precursors of other odour-active compounds (e.g., aldehydes, ketones, alcohols and shorter-chain carboxylic acids), which are produced during the ripening stage [34]. The key differentiating acids, based on weight, include dodecanoic acid, heptanoic acid, nonanoic acid, hexadecanoic acid, dec-9-enoic acid, (Z)-hexadec-9-enoic acid, tetradecanoic acid and (9Z,12Z)-octadeca-9,12-dienoic acid. Carrapiso et al. [74] found that in relation to the sensory profile and the fatty acid composition of subcutaneous fat, palmitic and (Z)-octadec-9-enoic acids were significantly correlated with a wide range of sensory traits. Furthermore, notable correlations were observed between either octadecanoic or (Z)-Octadec-9-enoic acids and various sensory attributes, such as brightness, juiciness, sweetness, fat *H* and cured aroma of Iberian dry-cured ham [75]. The aforementioned discriminating compounds are also present in various other types of dry-cured hams, including *Kraški pršut* [21], Parma [65], Toscano [70, 71], Iberian [68] and others.

Alcohols, primarily generated from lipid oxidation [14, 76], are crucial to the aroma profile of meat products, with herbaceous, woody, ham, vanilla and fatty notes [61, 62]. The key differentiating acids, based on weight, include oct-1-en-3-ol, hexan-1-ol, octan-1-ol, (E)-oct-2-en-1-ol, bis(2-butoxyethyl) hydrogen phosphate and 4-methylphenol. These compounds were also found in *Kraški pršut*, Parma, Iberian, Jamón Serrano, Toscano, Croatian, Istrian and Xinjiang mutton ham [11, 15, 21, 29, 60, 65, 71, 72]. Despite having higher threshold values than aldehydes, alcohols like oct-1-en-3-ol, with its low threshold [15], contribute significantly to the flavour profile, particularly with an intense mushroom aroma [29]. Hexan-1-ol, potentially derived from (Z)-hexadec-9-enoic acid and (Z)-octadec-9-enoic acid [71, 72], is recognised as a major volatile compound in meat [77]. Bis(2-butoxyethyl) hydrogen phosphate contributes to aromatic odour, fatty, oily, walnut and burnt notes [71]. Then, 4-methylphenol, although typically associated with

smoked products [15], is also present in *Kraški pršut*, which is not smoked during production. Nonetheless, the possibility of cross-contamination cannot be excluded, as the production facility may also handle other smoked products, potentially resulting in the unintentional transfer of smoke-derived compounds.

Ketones are primarily generated through lipid autoxidation and microbial metabolism ( $\beta$ -oxidation) of lipids [71]. These compounds exhibit relative stability in nature and are responsible for floral aroma [12]. During the salting stage of processing, ketone content increases, followed by a decline in subsequent stages. This pattern suggests that some ketones are transformed into acids and other volatile compounds during fermentation and ripening. These findings indicate that ketones are essential flavour precursors in the development of the characteristic aroma of dry-cured ham [11, 60]. A key differentiating ketone, based on weight and muscle type, is 6-methylhept-5-en-2-one. This compound is produced through  $\beta$ -keto decarboxylation or  $\beta$ -oxidation of saturated fatty acid [71] and is also found in *Kraški pršut*, Toscano and Iberian dry-cured ham [21, 71, 72, 78].

Esters are formed through the enzymatic esterification of fatty acids and alcohols during curing through the action of microorganisms such as lactic acid bacteria and *Micrococccae* [60]. In dry-cured hams, the origin of esters is most likely the esterification of carboxylic acids and alcohols generated by lipid oxidation in the intramuscular tissue [18]. Their low odour threshold contributes to the overall aroma, hence conferring dry-cured ham a fruit and sweet flavour [11], and has been associated with ripened flavour in cured meat products [79]. The key differentiating ester, based on weight, was ethyl (E)-hexadec-9-enoate. Yeasts, such as *Saccharomyces cerevisiae*, exhibit significant lipolytic and proteolytic activities, promoting the synthesis of medium-chain fatty acid ethyl esters, which impart pleasant fruity and floral aromas [80, 81]. [82] reported that the addition of *S. cerevisiae* is positively related to the contents of esters, alcohols, ethanol and total VOCs and consequently improves the sensory property.

Furans are formed through Maillard reactions and have very low odour thresholds contributing aromas such as sweet [17], cocoa, butter and fruit [12]. These compounds significantly enhance the characteristic aroma of dry-cured ham products [83]. A key differentiating furane, based on weight, is 5-pentylloxolan-2-one. This compound imparts distinctive cocoa, butter or fruit-like aromas [84] and has been found in various types of ham, including Toscano, Croatian varieties, Iberian and Xinjiang dry-cured mutton ham [11, 12, 29, 70, 71]. Another significant compound is 2-pentylfuran, derived from (9Z,12Z)-octadeca-9,12-dienoic acid and other n-6 fatty acids. It has a low perception threshold and a vegetable-like aromatic note. In addition to indicating lipid oxidation, 2-pentylfuran plays a crucial role in the overall flavour profile of ham [11].

An analysis of colour, texture and chemical parameters indicates that hams from the heavy and light weight groups are relatively similar. Within the light group (K\_light and SI\_light), the samples exhibit greater homogeneity, whereas a comparison of the heavy groups (F\_heavy and SI\_heavy)

shows complete separation (100%). This suggests that the raw material used for light hams was considerably more uniform than that for heavy hams, as substantial differences were observed in the final product. In terms of sensory attributes, the heavy groups further confirm that the raw materials from Slovenian and Italian suppliers differ significantly. In contrast, the light ham group shows less differentiation, implying that raw materials from Slovenia and Hungary are more similar. The LDA analysis of VOCs clearly distinguishes F\_heavy as the most distinct group. VOCs may also be useful as markers of quality or as indicators of defects, such as off-flavours, rancidity and related issues. For example, the compound hexanal is an important indicator of lipid oxidation; although it contributes to the overall aroma of ham [71], when present at high concentrations, it may indicate unpleasant, rancid and pungent flavour notes in dry-cured ham [85]. Similarly, higher levels of compounds, such as acetic acid, hexanoic acid, 3-methylbutanoic acid or 3-hydroxybutan-2-one, have been associated with defective dry-cured ham [85]. The OPLS-DA comparison further highlights the significant differences between SI\_heavy and F\_heavy. Based on these findings, it can be inferred that the Hungarian raw material used for light hams is more similar to the Slovenian raw material. In contrast, the Italian raw material for heavy hams consistently deviates across all analyses, which is reflected in the final product quality.

## 5. Conclusions

This study demonstrated the significant influence of weight categories and supplier variability on the quality of *Kraški pršut*, a traditional Slovenian dry-cured ham with PGI status. Sensory analysis indicated greater softness, juiciness, solubility, marbling and reduced saltiness in heavy hams compared to light hams. Texture analysis confirmed that heavy hams had lower *H*, *G* and *Ch*, attributed to higher  $a_w$  and moisture content. Supplier variability was evident, with Slovenian heavy hams receiving the highest sensory ratings, whilst Italian heavy hams showed greater variability and a slight tendency towards pastiness. Light hams from Slovenian and Hungarian suppliers were more similar in quality. VOC analysis further differentiated weight groups and suppliers, achieving high classification accuracy. The results of this study have important implications for the industry. Ensuring consistent raw material quality is crucial for achieving uniform product characteristics, particularly in the heavy ham category where variability was most pronounced. These findings suggest clear opportunities for improving process consistency and final product quality through more strategic selection of raw materials. For example, choosing suppliers that provide more uniform raw materials, such as those from Slovenia or Hungary for light hams, may help reduce variability. In addition, processing techniques, such as adjustments in salting, could be adapted to the specific characteristics of raw materials from each supplier. This is particularly relevant for Italian heavy hams, which showed the greatest variability. We recommend that the industry pay closer attention to the raw material,

especially to use for heavy hams, to minimise quality inconsistencies in the final product. Such efforts could improve product uniformity and enhance overall quality.

## Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

## Disclosure

This research is also part of the ISO-FOOD Centre and the METROFOOD-RI infrastructure.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Author Contributions

**Katja Babič:** writing – original draft and editing, conceptualisation, investigation, methodology, data curation, formal analysis. **Lidija Strojnik:** data curation. **Martin Škrlep:** conceptualisation, investigation, methodology, writing – review and editing. **Marjeta Čandek-Potokar:** conceptualisation, investigation, methodology, writing – review and editing, funding acquisition. **Nives Ogrinc:** writing – review and editing, conceptualisation, supervision, project administration, funding acquisition. All authors contributed to the article and approved the submitted version.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*) Supporting Information is available from the Wiley Online Library or from the author. The supplementary material provides an in-depth understanding of the physicochemical, sensory and volatile profile of Kraski pršut, along with statistical validation of differences amongst samples. It consists of the following components. Table S1: Colour properties in dry-cured hams. Table S2: Chemical properties in dry-cured hams. Table S3: Texture parameters in dry-cured hams. Table S4: Sensory evaluation of dry-cured hams. Table S5: Relative mean percentages of the volatile organic compounds identified in the dry-cured ham samples (K\_light, SI\_light, SI\_heavy

and F\_heavy) by headspace solid-phase microextraction gas chromatography–mass spectrometry. Figure S1: OPLS-DA score plots (a) and VIP values (b) in the pairwise comparison of heavy and light dry-cured ham groups by colour, texture and chemical analysis. Samples are colour-coded according to their weight class. The ellipse on the score plot denotes the 95% confidence interval. A red dashed line indicates the threshold used to identify the most important variables. Accuracy  $(TP + TN)/(TP + FP + FN + TN)$  and  $F1$  score ( $F1 \text{ score} = 2 * (\text{Recall} * \text{Precision})/(\text{Recall} + \text{Precision})$ ) of the model were calculated: Recall =  $TP/(TP + FN)$  and Precision =  $TP/(TP + FP)$  where TP is a true positive, TN a true negative, FP a false positive and FN a false negative. Accuracy is the ratio of the correctly labelled subjects (samples) to the whole pool of subjects (samples), and the  $F1$  score is the harmonic mean (average) of the precision and recall. Figure S2: OPLS-DA score plots (a) and VIP values (b) in the pairwise comparison of heavy and light dry-cured ham groups by sensory parameters. Samples are colour-coded according to their weight class. The ellipse on the score plot denotes the 95% confidence interval. A red dashed line indicates the threshold used to identify the most important variables. Figure S3: OPLS-DA score plots (a) and VIP values (b) in the pairwise comparison of Slovenian heavy and light dry-cured ham groups by sensory parameters. Samples are colour-coded according to their weight class. The ellipse on the score plot denotes the 95% confidence interval. A red dashed line indicates the threshold used to identify the most important variables. Figure S4: OPLS-DA score plots (a) and VIP values (b) in the pairwise comparison of heavy and light dry-cured ham groups by VOCs profile. Samples are colour-coded according to their weight class. The ellipse on the score plot denotes the 95% confidence interval. A red dashed line indicates the threshold used to identify the most important variables. Figure S5: OPLS-DA score plots (a) and VIP values (b) in the pairwise comparison of Slovenian heavy and light dry-cured ham groups by VOCs profile. Samples are colour-coded according to their weight class. The ellipse on the score plot denotes the 95% confidence interval. A red dashed line indicates the threshold used to identify the most important variables. Figure S6: Discriminant function score plot (a) and a discriminant loadings plot (b) for ham samples from different suppliers F\_heavy ( $n = 8$ ), K\_light ( $n = 8$ ), SI\_heavy ( $n = 8$ ) and SI\_light ( $n = 8$ ). Figure S7: OPLS-DA score plots (a) with VIP values in the pairwise comparison between different suppliers by colour, texture and chemical analysis. (b) Heavy group—SI\_heavy versus F\_heavy. (c) Light group—SI\_light versus K\_light. Samples are colour-coded according to their weight class. The ellipse on the score plot denotes the 95% confidence interval. A red dashed line indicates the threshold used to identify the most important variables. Figure S8: Discriminant function score plot (a) illustrates the spatial separation of sensory profiles between different suppliers, whilst the discriminant loadings plot (b) depicts the relative contribution of each sensory attribute to the discrimination. F\_heavy ( $n = 8$ ), K\_light ( $n = 8$ ), SI\_heavy ( $n = 8$ ) and SI\_light ( $n = 8$ ). OPLS-DA score plots (c for heavy groups and d for light groups) in the pairwise comparison between different suppliers by sensory evaluation. Samples are colour-coded according to



their weight class. The ellipse on the score plot denotes the 95% confidence interval. A red dashed line indicates the threshold used to identify the most important variables.

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