

Research Article

# Sensory and Nutritional Characterization of Six Different Types of Croatian Traditional Meat Product Characterization of Croatian Traditional Meat Products

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The aim of this study was to determine quality parameters of Croatian traditional meat products (TMPs) via the analysis of their sensory and nutritive parameters, including physicochemical properties, fatty acid profile, mineral content, and amino acid composition. Over 2020 and 2021, a diverse collection of 242 dry-fermented sausages and dry-cured TMPs from various rural households was assessed. Sensory evaluations highlighted their satisfactory qualities, with moderate salinity and a unique taste profile characterized by a slight bitterness, with low sweetness and sourness levels. Detailed analyses revealed significant differences ( $p < 0.05$ ) across samples in key parameters such as water activity, pH, moisture, ash, fat, protein, carbohydrate, and NaCl content, as well as in the fatty acid profile and mineral content, underscoring the diversity and richness of these products. Amino acids such as alanine, histidine, isoleucine, leucine, and proline were present in high amounts in all samples, largely varying in their content within the same product type. The findings underscore the high quality and distinctiveness of Croatian TMPs, attributed to varied recipes and production techniques across different regions, suggesting the potential for standardization and further quality enhancement. Despite the presence of saturated fats and salt, the nutritional value of these TMPs, marked by high protein content and essential minerals, aligns with health-conscious consumer trends, presenting an opportunity for increased global appreciation of these artisanal products. The positive results from the sensory analysis underscore the TMPs' ability to align with consumer taste preferences, thereby affirming their reputation as a national delicacy. This could represent a promising direction for future research, focusing on the refinement of production and marketing strategies to attract a global audience, especially those with a preference for health-conscious and traditional culinary options.

## 1. Introduction

Globalization and modern technological processes in the production of meat products have led to a uniformity that threatens the distinct identity of individual countries. Traditional meat products (TMPs) embody the cultural heritage of a nation, representing unique and recognizable products from specific regions, primarily manufactured by small, local producers. According to Laranjo et al. (2017) [1], TMPs are valued for their sensory and nutritional qualities, derived from time-honoured domestic recipes. To promote and protect these unique TMPs, known for their organoleptic diversity and nutritional value, they are often labelled as protected designation of origin (PDO), protected geographical indications (PGI), and/or traditional specialities guaranteed (TSG) products [2]. Among the TMP samples in this study, *Pršut*, *Kulen*, pancetta, and *Pečenica* have been awarded the PGI or PDO label. The European Union, with significant contributions from Germany, Italy, Spain, and France, leads in the production of fermented meat products, accounting for 20–40% of all processed meats [3–5]. Croatia, with its unique geographical and climatic features, produces a variety of TMPs, such as dry-cured hams, loins, sausages, and bacon, each with a distinct brand identity [6]. The diversity in recipes for similar meat products, like ham, varies significantly across different regions, influenced by local climate conditions, which affect the drying and curing processes. These factors, in turn, impact the chemical composition and, notably, the sensory profiles of the meat products [7].

TMPs are considered as important dietary source of proteins, fats, minerals, fatty acids, and essential amino acids. However, their high content of saturated fats and salt can pose health risks when consumed excessively [8]. Pork-based products generally contain a higher proportion of saturated (SFA) and a lower proportion of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids [9]. Holk et al. [8] state that the total intake of fat should not exceed 30% or 10% of SFA in total energy intake. While the salt content in meat products is crucial for taste, texture, and microbiological safety, excessive consumption (above 5 g/day) can lead to hypertension and increased heart disease risk [8]. The World Health Organization (WHO) (2023) [10] state that reducing salt intake is the most cost-effective way to improve the health of the population. Despite these concerns, TMPs, with their well-balanced nutritional profile, can be part of a healthy diet if consumed in moderation (2–3 times a week) by healthy individuals [11].

TMPs are also rich in minerals, especially Fe and Zn, and contain significant amounts of K and Mg [2, 12] needed for the proper growth and development of the human body. During the production that involves different technological processes, TMP lipids undergo transformation that includes hydrolytic processes, the release of short-chain fatty acids, and oxidation, resulting in the formation of volatile components, and specific aromas responsible for the characteristics of the final products [13–15]. In addition, microbiota involved in ripening and fermentation processes, and moulds overgrowing TMPs, affect their sensory

properties [16, 17], especially their appearance, taste, and smell. Onwards, to ensure uniform sensory properties, the meat used as the major raw material should be of the highest quality [2]. Despite the popularity of European TMPs among consumers for their unique organoleptic characteristics and quality, concerns regarding their uniformity, health safety, and nutritional aspects persist. Recent review by Halgarda and Wójciak [2] highlights these issues, emphasizing the need for careful consideration of moisture, protein, salt, fat content, and fatty acid profiles in these products.

This study aims to investigate the nutritive and sensory characteristics of the main representatives of Croatian TMPs, produced in different areas and categorized into six categories. It marks the first comprehensive investigation into the physicochemical properties, fatty acid profiles, mineral content, and amino acid composition of these products, alongside a sensory analysis of a large sample size ( $n = 242$ ). This research not only offers an extensive characterization of these products' most important attributes but also emphasizes TMPs as an integral part of Croatia's cultural heritage, covering all dry-cured meat products traditionally produced in the region. By offering a detailed overview of their most important attributes, this research not only contributes to the understanding of Croatian TMPs but also supports efforts towards standardization and improved global recognition of these value-added products.

## 2. Materials and Methods

**2.1. Traditional Meat Product Samples.** A total of 242 TMPs, out of which 99 dry-fermented sausages and 143 dry-cured meat products, were sourced from various Croatian regions (northern, central, western, eastern, and southern), each distinguished by its unique climate and cultural heritage. These samples were collected from local fairs and directly from family farms (OPGs) in quantities ranging from 1000 to 1500 grams. The sampling spanned a two-year period (2020–2021), with efforts made to ensure consistency in the quantity and type of samples from the same locations across both years, whenever possible. For the products collected in 2020, the ripening process spanned from 2019 to mid-2020; similarly, for those collected in 2021, ripening occurred from 2020 to the first half of 2021. All TMPs were produced by rural households adhering to traditional recipes and methodologies. The products under study included six different types of TMPs, as follows: *Kulen/Kulenova seka* ( $n = 34$ ) and other dry-fermented sausages ( $n = 65$ ) produced according to the previously described recipes [16–19], as well as prosciutto/dry ham ( $n = 42$ ), dry neck/*Budola* ( $n = 22$ ), dry loin/*Pečenica/Ombolo/Kare/Zarebnjak* ( $n = 29$ ), and bacon/pancetta ( $n = 50$ ), whose production process was also described earlier [12, 18, 20–22]. Shortly, *Kulen/Kulenova seka* and dry-fermented sausages are made from lean pork meat and fat by grinding, mixing with salt and spices, and filling into natural pork casings. These products are subjected to fermentation and smoking at 15–20°C, and afterwards dried and ripened at 12–16°C. Because of the differences in the diameter of the casings they are filled into, the production of dry sausages takes at least 30 days, that of *Kulenova seka*

more than 60 days, and that of *Kulen* more than 120 days. Prosciutto, dry ham, dry neck, dry loin, and bacon are produced in the manner typical for dry-cured products, which implies salting at temperatures lower than 5°C, cold smoking, drying, and ripening at temperatures around 15°C. The main difference between these products is the duration of their production, varying from two months for dry loins, over three to four months for dry neck and bacon, up to more than 12 months for prosciutto and dry hams. Furthermore, in the production of Istrian prosciutto (western region), the skin and subcutaneous fatty tissue are firstly removed from the meat, which is a difference compared to other regions. Also, the traditional production of Istrian prosciutto requires processing of the fresh ham in such a way as to leave the pelvic bones intact, which is a unique characteristic worldwide and contributes significantly to its visual appeal. The western region is characterized by drying with natural ventilation, while the southern region is known for cold smoking on rainy and warmer days and exposure to drafts and drying during cold northern wind [23]. Notably, no nitrites/nitrates, acidifiers, antioxidants, or starter cultures are added to these products [23]. This practice aligns with traditional production methods aimed at preserving their authenticity and cultural significance. It also offers consumers a product that is healthier, more flavourful, and environmentally sustainable when considering the entire production process. The TMP samples were firstly used for sensory evaluation and then chopped into small pieces and homogenized using a Grindomix GM 200 apparatus (Retsch, Germany) according to the ISO 3100-1:1975 [24] (sampling and preparation of test samples). After homogenization, colorimetric parameters were determined. The homogenates were stored in a refrigerator at +4°C, which is the typical temperature for storage of dry-cured meats or fermented sausages, pending determination of physicochemical properties, mineral and fatty acid content, and amino acid composition.

**2.2. Determination of Sensory Characteristics.** Sensory evaluation of TMP samples was performed by the ISO 8589:2007 [25] by nine highly trained panellists. The sensory analysis was conducted over two years in eight sessions in the sensory laboratory of the Faculty of Food Technology and Biotechnology, University of Zagreb. The room-related technical requirements included a relative humidity of 50%–55% and a temperature of 20–22°C, with illumination set at 4000 K and 500 lux provided for the working table. Sensory evaluation utilized a quantitative descriptive analysis (QDA) based on a numerical and unipolar intensity scale. The intensity of each sensory property was estimated using a numerical scale calibrated from left to right, with “0” indicating the absence of a given sensory trait and “9” indicating its strongest intensity. The evaluated properties were visual and olfactory qualities, texture, mouth feel, and aroma of the product. Detailed descriptions of sensory traits, definitions, and the range implemented for sensory evaluation are presented in the study by Lešić et al. [16].

Individually coded samples were served at room temperature (two 2-mm-thick slices) in sensory booths. Samples were presented monadically in a randomized order, with a 5-minute break considered between samples. Replicated samples were used to calculate the panellist's repeatability. Water, yogurt, sour apple, and unsalted bread were provided to assessors between samples as palate cleansers.

**2.3. Determination of Physicochemical Properties and Fatty Acid Profile.** The colour of the homogenized TMP samples was determined in accordance with the CIE (Commission Internationale de l'Eclairage)  $L^* a^* b^*$  colour system using a Chroma Meter CR-400 SET Colorimeter (Konica Minolta, Tokyo, Japan) at room temperature. Before the analysis, the colorimeter was calibrated using a white calibration plate (D65;  $Y = 85.6$ ,  $x = 0.3183$ ,  $y = 0.3357$ ).

All chemicals used for the analyses were of an analytical grade. Moisture content was determined gravimetrically using the standard ISO 1442:1997 analytical method [26] and a thermostat (UF75 plus Memmert, Germany) by virtue of sample drying at 103°C to achieve constant weight and obtain crude ash (ISO 936:1998) [27] by burning the TMP samples in a muffle furnace (LV9/11/P320, Nabertherm, Germany) for 6 hours at  $550 \pm 25^\circ\text{C}$ . The total protein content was determined by the Kjeldahl method (ISO 937:1978) [28] using a digestion block (Unit Gerhardt, Germany) and an automated distillation and titration device (Vapodest 50 s, Foss, Denmark). Total fats were determined by the Soxhlet method (ISO 1443:1999) [29], the fats thereby being extracted using petroleum ether and an extraction device (Soxtherm 2000, Gerhardt, Germany). pH values were measured using a MP220 pH meter (Mettler Toledo, Switzerland), while the water activity ( $a_w$ ) was measured using an HC2-AW probe (IKA-Rotronic, Switzerland).

Fatty acid methyl esters were prepared from the extracted fats according to the ISO 12966-2:2015 [30], using hexane as solvent and 2N methanolic potassium hydroxide solution for transmethylation. The prepared methyl esters were analysed using gas chromatography according to the ISO 12966-4:2015 [31] and a gas chromatograph with flame ionization detector 7890B (Agilent Technologies, USA) having a 60-m-long DB-23 capillary column measuring 0.25 mm in diameter, the layer thickness thereby being fixed at 0.25  $\mu\text{m}$  (Agilent Technologies, USA). The procedure was described in detail earlier [6, 32, 33].

**2.4. Determination of Mineral Content.** After acid digestion of ~0.2 g of a sample with 7 mL of 60% nitric acid and 1 mL of 30% hydrogen peroxide in a microwave oven (Ethos easy, Milestone, Italy), TMP samples were transferred into (25 mL and 50 mL) measuring flasks and supplemented with water up to the mark. Determination of sodium (Na), calcium (Ca), potassium (K), magnesium (Mg), copper (Cu), zinc (Zn), and iron (Fe) was performed by flame atomic absorption spectroscopy (200 Series A4 with SPS 4 Auto-sampler, Agilent Technologies, USA) at  $\lambda = 589.0$  nm for Na,  $\lambda = 422.7$  nm for Ca,  $\lambda = 766.5$  nm for K,  $\lambda = 285.2$  nm for

Mg,  $\lambda = 324.8$  nm for Cu,  $\lambda = 213.9$  nm for Zn, and  $\lambda = 248.3$  nm for Fe. For each mineral, an HC-coded lamp specific for the given mineral (Agilent Technologies, SAD) and standard 1,000  $\mu\text{g/mL}$  solution in 5% nitric acid (Agilent Technologies, USA) was used.

**2.5. Determination of Amino Acid Composition.** For sample preparation and derivatization of free amino acids (FAAs), the method of Kritikos et al. [34] was used with some modifications. Briefly, 1,000 g of a previously homogenized sample (Retsch GM 200, Retsch, Germany) and 50  $\mu\text{L}$  of the internal standard norleucine (50 mg/L) were weighed into a Falcon centrifuge, supplemented with 0.1 M HCl up to 30 g, and homogenized with an Ultra-Turax T-25 digital with S 25 N-18 G dispersing tool (IKA, Germany) for 1 min at 14,000 rpm. The samples were then centrifuged at 4,500  $\times$  g for 10 min (Hettich 320 R, Germany). At a later time, 100  $\mu\text{L}$  of the supernatant was transferred into a reaction vial and 120  $\mu\text{L}$  of 0.33 M sodium hydroxide was added, followed by the addition of 80  $\mu\text{L}$  of 23% (v/v) picoline in n-propanol and 50  $\mu\text{L}$  of n-propyl chloroformate in chloroform/isooctane solution (composition: 17.4/71.6/11, v/v/v). The solutions were then vortexed for 1 min, allowed to rest for 2 min, and then vortexed for another minute.

Derivatives were acquired through liquid-liquid extraction (LLE) after adding 400  $\mu\text{L}$  of ethyl acetate to form a two-phase solvent system. Samples were vortexed for 1 min, left to rest for 5 min, and vortexed again for 1 min before the two layers were separated. The organic phase was evaporated for 15 min under a light stream of nitrogen at the temperature of about 35°C. Five hundred microlitres of methanol/water mixture 3/1 (v/v) containing 1 mM ammonium formate was used for reconstitution.

For the determination of concentrations of free amino acid derivatives, a reversed-phase UPLC-MS/MS analysis was used for separation and quantification. The UPLC system used was ACQUITY™ UPLC™ H-Class PLUS (Waters, CA, USA), which consists of a thermostatted FTN sample manager, a thermostatted column compartment, and a quaternary solvent manager. The UPLC system was coupled with a Xevo TQ-S micro triple quadrupole mass spectrometer (Waters, Milford, MA, USA). Chromatographic separation was performed using a Kinetex C18 column (1.7  $\mu\text{m}$ , 150 mm  $\times$  2.1 mm; Phenomenex) in the following settings: column temperature 35°C, injection volume 1  $\mu\text{L}$ , and mobile phase flow rate 250  $\mu\text{L}/\text{min}$ . The mobile phase components were 1 mM ammonium formate in water (solution A) and 1 mM ammonium formate in methanol (solution B). The mobile phase gradient was programmed as follows: 0.00–8.00 min, 60–83% B; 8.00–8.10 min, 83–60% B; and 8.10–12.00 min, 60% B.

Mass spectrometer was operated in the positive ionization mode (ESI+), the performance parameters being as follows: electrospray capillary voltage, 1.50 kV; cone voltage, 30 V; extractor voltage, 2 V; source temperature, 150°C; desolvation temperature, 500°C; cone gas flow, 100 L/h, desolvation gas flow, 800 L/h; and collision energy, 20 eV. The MRM transitions are listed in Table 1. Data signals were

TABLE 1: MS parameters of derivatized amino acids.

Daughter ion ( $m/z$ )	Molecular ion of derivate ( $m/z$ )	Amino acid (abbreviation)
75.81	204.20	Glycine (GLY)
172.00	275.29	Glutamine (GLN)
160.00	248.20	Threonine (THR)
130.00	218.20	Alanine (ALA)
190.00	336.30	Cysteine (CYS)
141.88	278.30	Methionine (MET)
170.00	361.20	Lysine (LYS)
158.00	246.20	Valine (VAL)
172.00	318.20	Glutamic acid (GLU)
245.00	333.30	Tryptophan (TRP)
172.00	260.20	Leucine (LEU)
130.00	260.20	Isoleucine (ILE)
206.00	294.30	Phenylalanine (PHE)
136.00	396.20	Tyrosine (TYR)
103.73	234.20	Serine (SER)
156.00	244.20	Proline (PRO)
172.10	260.20	Hydroxyproline (HYP)
156.00	303.20	Arginine (ARG)
196.00	370.20	Histidine (HIS)
216.12	304.20	Aspartic acid (ASP)
216.00	260.20	Norleucine (nLEU)

acquired and processed using the MassLynx Software (V4.2 SCN1035; 2022, Waters).

The FAA identification was performed by comparing the retention time and mass spectrometric data of amino acid standards (Supelco, A9906) and norleucine (Sigma-Aldrich, N8513) as the internal standard; the quantification was based on the comparison between the peak areas and the internal standards.

**2.6. Data Analysis.** The evaluation of panellists' performance included assessing repeatability (based on the absolute differences between the scores assigned to the different descriptors of the sample and its replica), reliability (based on the absolute differences between the scores assigned by each panellist to the different descriptors of the various samples compared to the median scores of the panel), and discriminant capacity (the ability to detect differences among samples regarding each descriptor). This evaluation was conducted within the Big Sensory Soft software (Centro Studi Assaggiatori, Brescia, Italy). Results were graphically presented as mean value for  $n$  number of samples according to the group of products. Statistical analysis was performed using the SPSS Statistics Software 22.0 (SPSS Statistics, NY IBM, 2013, Sankt Ingbert, Germany). For all physico-chemical properties, results are presented in tables and graphically as mean  $\pm$  SD for  $n$  number of samples according to the group of products. The differences between the sample groups were established using the analysis of variance (ANOVA) with either the Tamhane or the Scheffe post hoc test, depending on the variance analysed by the Levene test, with the statistical significance set at 95% ( $p = 0.05$ ). In the analysis of the data from the sensory analysis, the product type was considered a fixed effect, while the panellist was considered a random effect.

The obtained results were subjected to the principal component analysis (PCA) on the correlation matrix. In this context, PCA facilitated the understanding of the relationships between sensory attributes, physicochemical traits, fatty acids, amino acids, and mineral content. By examining the loadings of each variable on the principal components, insights were gained into which factors contributed most significantly to the overall variation observed in the dataset. Moreover, PCA on the correlation matrix is particularly suitable for analysing datasets with variables measured on different scales or with varying units of measurement. By standardizing the variables to have a mean of zero and a standard deviation of one, PCA on the correlation matrix ensures that all variables contribute equally to the analysis, regardless of their original scales.

### 3. Results and Discussion

**3.1. Sensory Characteristics.** TMP ripening includes physicochemical changes, proteolytic and lipolytic reactions, and surface mould growth, which also affects sensorial properties typical of the final product [13, 16]. Sensory properties play an important role in the final decision on the acceptability of a meat product [35]. Sensory profiles of TMPs determined in this study are shown in Figure 1. It was found that 16 out of 26 evaluated objective sensory characteristics (colour of the muscle tissue, colour uniformity, fat content, surface humidity, juiciness, saltiness, sweetness, sourness, bitterness, spiciness, hotness, buttery aroma, aromatic herbs, spice herbs, off-flavours, and mouldy aroma) were statistically significantly different ( $p < 0.05$ ), while no statistically significant difference was found in the remaining 10 evaluated properties (colour of the adipose tissue, cohesiveness, marbling, tyrosine crystals, favourable odours, unfavourable odours, smoky odour, tenderness, ripen meat aroma, and fresh pork meat aroma). Even the visible differences in the studied properties listed above mirror the diversity of technological production procedures and spices and recipes used by the producing households, typical of the given Croatian production region [36].

The obtained results point to the variability of colour uniformity of the studied products depending on the type of product and correlating to the colour of the muscle tissue and the amount of fat used in the production. Water and fat content are correlated with tenderness and juiciness of the product [36]. The salinity of TMPs was characterized as moderate (4.09–6.02), with low values for sweetness ranging from 1.2 for *Kulen/Kulenova seka* to 1.63 for prosciutto/dry ham. The sourness values ranged from 1.01 for *Pečenica/Ombolo/Kare/Zarebnjak* to 2.7 for *Kulen/Kulenova seka*, while the bitter taste ranked from 0.77 for *Pečenica/Ombolo/Kare/Zarebnjak* to 2.44 for dry neck/*Buđola*. The aroma given by aromatic herbs was more pronounced in *Pečenica/Ombolo/Kare/Zarebnjak*, while the spicy aroma coming from spice herbs was most intense in *Kulen/Kulenova seka* and dry-fermented sausage. The mouldy aroma was more pronounced in prosciutto/dry ham, since during ripening, their surface gets abundantly covered with moulds able to produce odour-active compounds [20]. Ruiz et al. [37] and

Kos et al. [7] stated that juiciness as a textural property, bitterness as a taste-related property, and aroma play a significant role in the acceptability of the product adjudicated by consumers. Furthermore, odour and aroma are most important for characterizing and differentiating dry-cured meat products [7]. In this study, all analysed TMPs showed good acceptability (Figure 1(b)), with values around 7, and had similar subjective sensorial characteristics like taste, smell, cross-section and consistency attractiveness, steadiness, and richness of favourable aromas. Similar results were published by Lešić et al. [20] for traditional sausages, in which moulds of the *Penicillium genera* (*P. nalgiovense*, *P. solitum*, and *P. commune*), which have desirable effects on sensorial properties, were isolated very frequently. Furthermore, this research can be compared to the previous studies on Croatian TMPs [7, 20, 36], whose authors stated that TMPs produced in rural households have intense aromas, high salinity, distinctive taste, and slightly pronounced spiciness and bitterness. In addition, Kos et al. [7] claimed that in defining a sensory profile of a TMP, the production region plays an important role.

Appearance, especially the colour of a meat product, is very often the main quality-assessing criterion that significantly affects consumer preferences [20, 38]. The colour of TMPs is affected by natural meat pigments, muscle structure, fat-lean ratio, type and quantity of spices, and technological production process [20, 39, 40]. Colour measurement results, i.e., CIE  $L^*a^*b^*$  values, are shown in Table 2. TMP lightness ( $L^*$ ) values ranged from 40.94 for *Kulen/Kulenova seka* to 53.74 for bacon/pancetta, while the redness ( $a^*$ ) marks ranged from 5.19 for bacon/pancetta to 14.93 for *Kulen/Kulenova seka*. The yellowness ( $b^*$ ) values ranged from 8.70 for dry neck/*Buđola* to 15.06 for *Kulen/Kulenova seka*. Except for lightness ( $L^*$ ), other colour parameters were found to be significantly different, but our results are similar to the previous literature data on traditional sausages [20, 39], although in this study, prosciutto/dry ham  $a^*$  and  $b^*$  values higher than those determined by Marušić Radovčić et al. [40] were established. In this research, the highest redness values were obtained for *Kulen/Kulenova seka* and dry-fermented sausages, which is the result of the addition of red pepper during production. Onwards, high yellowness values can also be linked to the yellow carotenoids found in the peppers typically added to *Kulen/Kulenova seka* [39]. Research by other authors shows that the decrease in redness is attributed to the lower proportion of proteins, while a low proportion of fat and a high proportion of water increase the redness and decrease the lightness values [20, 39].

**3.2. Physicochemical Properties and Fatty Acid Profile.** Results of the analysis of physicochemical properties obtained for six different types of TMPs are presented in Table 3. The  $a_w$  value has an important role because it affects product safety and stability by controlling the growth of microorganisms and biochemical reactions [41]. The  $a_w$  values ranged from 0.82 for prosciutto/dry ham and bacon to 0.86 for *Pečenica/Ombolo/Kare/Zarebnjak*. The obtained  $a_w$

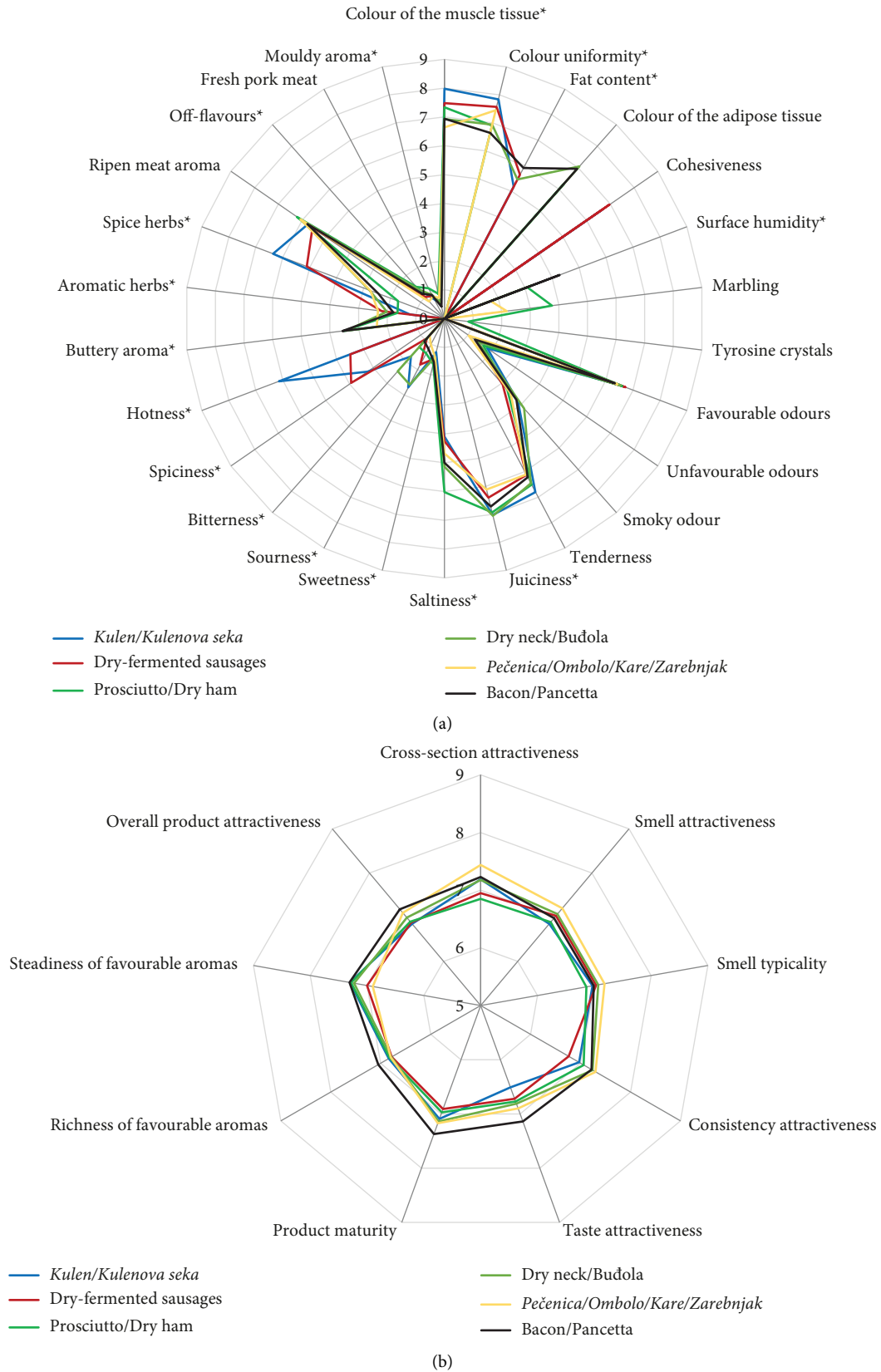


FIGURE 1: Sensory characteristics of traditional meat products displayed based on the product type: (a) objective characteristics; (b) subjective characteristics. \*a statistically significant difference ( $p < 0.05$ ).

TABLE 2: Colorimetric parameters displayed based on the type of a traditional meat product.

TMP	$L^*$ lightness	$a^*$ redness	$b^*$ yellowness
<i>Kulen/Kulenova seka</i> ( $n = 34$ )	40.94 ± 3.74	14.93 ± 2.72 <sup>a</sup>	15.06 ± 3.74 <sup>a</sup>
Dry-fermented sausages ( $n = 65$ )	41.26 ± 4.39	10.18 ± 2.80 <sup>ab</sup>	11.42 ± 3.56 <sup>bc</sup>
Prosciutto/dry ham ( $n = 42$ )	46.68 ± 5.02	8.58 ± 1.60 <sup>ab</sup>	10.55 ± 1.98 <sup>cd</sup>
Dry rack/ <i>Budola</i> ( $n = 22$ )	44.37 ± 3.45	8.39 ± 1.50 <sup>b</sup>	8.70 ± 1.59 <sup>d</sup>
<i>Pečenica/Ombolo/Kare/Zarebnjak</i> ( $n = 29$ )	52.52 ± 6.82	6.80 ± 2.13 <sup>b</sup>	10.11 ± 1.42 <sup>cd</sup>
Bacon/pancetta ( $n = 50$ )	53.74 ± 3.86	5.19 ± 2.38 <sup>b</sup>	10.72 ± 1.79 <sup>c</sup>

<sup>a-d</sup>values within the same row with no common superscript differ significantly ( $p < 0.05$ ).

results are in accordance with the previously published literature data relating to TMPs and claiming that the  $a_w$  values of the above products are  $\leq 0.9$  [8]. Ambrosiadis et al. [42] determined the  $a_w$  of Greek traditional sausages to be  $0.959 \pm 0.015$ , while Çiçek and Polat [14] found the  $a_w$  of *Bez Sucuk* (Turkish traditional sausage) to be in the range of 0.913–0.935, as opposed to Kaval et al. [43], who claimed it to be 0.774–0.979. Giovanelli et al. [44] determined the  $a_w$  of 0.873 for Tuscan, 0.928 for San Daniele, and 0.935 for Parma hams, and concluded that the final  $a_w$  values depend on NaCl concentration. The technology of Italian ham production is different from that exercised in Croatia, since the Italian hams are not smoked and pressed, and are coated with a mixture of lard, rice flour, and black pepper before drying, which reduces their dehydration during the process [45]. In Tuscan hams, the highest NaCl content (4.48%) was determined (as compared to 2.63% in San Daniele and 2.44% in Parma hams), which explains the lower  $a_w$  values found in them. The same was found also for the TMPs embraced by this study, since the lowest  $a_w$  value was determined in prosciutto/dry ham samples ( $0.82 \pm 0.05$ ), which had the highest salt content ( $6.58 \pm 1.88$  g/100 g).

During the TMP production, in specific in the ripening stage, physicochemical changes occur. Salt content, pH, moisture, and  $a_w$  are changing, which, in combination with biochemical changes (proteolysis and hydrolysis), affects taste, flavour, and texture of the final products. Fernández-Fernández et al. [46] and Kaval et al. [43] also stated that pH and  $a_w$  are affected by the processing conditions and recipes used. pH values of the analysed TMPs ranged from 5.42 for *Kulen/Kulenova seka* to 6.23 for bacon/pancetta. Similar pH values descriptive of traditional Croatian sausages were determined by Lešić et al. [20] (5.23 for *Kulenova seka*, 5.32 for Slavonian domestic sausage, 5.90 for *Kulen*, 5.94 for Istrian sausage, and 6.26 for Istrian domestic sausage). Martuscelli et al. [41] reported the pH value of dry-cured hams to range from 5.71 to 5.87 regardless of the salting process and the TMP class weight. Due to the low water content (<40%) found in all analysed samples except for *Pečenica/Ombolo/Kare/Zarebnjak* (45.80%), and high protein (20.75 to 38.34%) and fat content (8.97 to 46.95%), TMPs are recognized as high-quality meat products. The highest water content was measured in *Pečenica/Ombolo/Kare/Zarebnjak* ( $45.80 \pm 7.78\%$ ) and the lowest in bacon/pancetta ( $25.90 \pm 10.31\%$ ). Kovačević et al. [47] stated that production technologies used with dry-fermented products decrease water and increase ash, protein, and fat content of the products. In our study, the ash content was found to span

as follows: prosciutto/dry ham (8.24%) > dry neck/*Budola* (7.25%) > *Pečenica/Ombolo/Kare/Zarebnjak* (6.54%) > *Kulen/Kulenova seka* (5.52%) > bacon/pancetta (5.37%) > dry-fermented sausage (5.12%).

Similar ash content was determined in Dalmatian (7.87%) and Istrian ham (9.55%) by Kos et al. [12], in the research that dealt with the mineral composition of the two Croatian hams. Kovačević et al. [45] stated that the ash content in Slavonian ham ranges from 5.31 to 7.73%, while Pleadin et al. [33] also determined the highest ash content in prosciutto samples (9.81% in Croatian vs 7.77% in Montenegrin samples). In the same study, Pleadin et al. [33] determined lower ash values in domestic sausages (5.02% in Croatian vs 4.79% in Montenegrin sausages) and higher ash values in dry neck (8.19% in Croatian vs 6.72% in Montenegrin dry necks). Meat products' fat content varies depending on the raw material, i.e., the portion of meat used in the production, fatty tissue amount, and recipe used [47]. The highest fat content found in TMPs embraced by this study was that in bacon/pancetta samples ( $46.95 \pm 14.02\%$ ), while the lowest was determined in *Pečenica/Ombolo/Kare/Zarebnjak* ( $8.97 \pm 6.44\%$ ). The obtained values are in line with the previously published data on similar products [6, 20, 33, 36]. In the research conducted by Pleadin et al. on Croatian and Slovenian traditional meat products [6], fat content found in Istrian sausages was 44.0%, that in dry neck 31.20%, that in Dalmatian prosciutto 21.7%, and that in Kraški prosciutto 23.20%.

Figure 2 shows the share of fatty acids across various TMPs. These proportions were as follows: MUFA (44.54–50.10%) > SFA (39.70–48.75%) > PUFA (6.59–11.15%). Pleadin et al. [33] found a similar fatty acid profile typical of pork meat products when studying TMPs from Croatia and Montenegro (MUFA, SFA, and PUFA, 41.97–49.75%, 39.96–45.94%, and 7.69–14.96%, respectively). These products contain a high proportion of SFA, so that moderate intake has been advised to consumers [6], given that SFAs are responsible for the development of cardiovascular diseases. Therefore, the World Health Organization (WHO) [48] recommends that fat should provide between 15% and 30% of daily calories, the share of SFAs thereby being up to 10% [11, 49]. On the other hand, fatty acids are important for sensory properties of meat products, their tenderness, and shelf-life length [33].

Due to the high protein content, TMPs are considered to be highly nutritious food. The protein content span determined in this study reads as follows: *Pečenica/Ombolo/Kare/Zarebnjak* ( $38.34 \pm 7.98\%$ ) > *Kulen/Kulenova seka* ( $33.23 \pm 5.05\%$ ) > prosciutto/dry ham ( $32.52 \pm 5.84\%$ ) > dry-

TABLE 3: Results (mean  $\pm$  SD) of the analysis of physicochemical properties, displayed for each type of traditional meat products.

Parameter	<i>Kulen/Kulenova seka</i> ( <i>n</i> = 34)	Dry-fermented sausages ( <i>n</i> = 65)	Prosciutto/dry ham ( <i>n</i> = 42)	Dry neck/ <i>Budola</i> ( <i>n</i> = 22)	<i>Pečenica/Ombolo/Kare/Zarebnjak</i> ( <i>n</i> = 29)	Bacon/pančetta ( <i>n</i> = 50)
$a_w$	0.83 $\pm$ 0.04 <sup>b</sup>	0.84 $\pm$ 0.06 <sup>b</sup>	0.82 $\pm$ 0.05 <sup>b</sup>	0.86 $\pm$ 0.04 <sup>ab</sup>	0.88 $\pm$ 0.06 <sup>a</sup>	0.82 $\pm$ 0.07 <sup>b</sup>
pH	5.42 $\pm$ 0.38 <sup>a</sup>	5.56 $\pm$ 0.51 <sup>a</sup>	6.17 $\pm$ 0.26 <sup>b</sup>	6.07 $\pm$ 0.34 <sup>b</sup>	6.01 $\pm$ 0.36 <sup>b</sup>	6.23 $\pm$ 0.38 <sup>b</sup>
Moisture%	33.65 $\pm$ 5.38 <sup>c</sup>	30.22 $\pm$ 7.56 <sup>cd</sup>	38.86 $\pm$ 8.07 <sup>b</sup>	39.59 $\pm$ 6.70 <sup>ab</sup>	45.80 $\pm$ 7.78 <sup>a</sup>	25.90 $\pm$ 10.31 <sup>d</sup>
Ash%	5.52 $\pm$ 0.83 <sup>bc</sup>	5.12 $\pm$ 0.65 <sup>c</sup>	8.24 $\pm$ 2.20 <sup>a</sup>	7.25 $\pm$ 1.32 <sup>ab</sup>	6.54 $\pm$ 1.87 <sup>b</sup>	5.37 $\pm$ 1.24 <sup>bc</sup>
Fat%	26.11 $\pm$ 7.17 <sup>c</sup>	33.88 $\pm$ 7.48 <sup>b</sup>	19.61 $\pm$ 11.14 <sup>d</sup>	24.56 $\pm$ 8.34 <sup>dc</sup>	8.97 $\pm$ 6.44 <sup>e</sup>	46.95 $\pm$ 14.02 <sup>a</sup>
Protein%	33.23 $\pm$ 5.05 <sup>ab</sup>	29.33 $\pm$ 3.90 <sup>c</sup>	32.52 $\pm$ 5.84 <sup>a</sup>	27.88 $\pm$ 3.85 <sup>c</sup>	38.34 $\pm$ 7.98 <sup>b</sup>	20.75 $\pm$ 5.97 <sup>d</sup>
Carbohydrates %	1.49 $\pm$ 1.03 <sup>a</sup>	1.47 $\pm$ 1.35 <sup>a</sup>	0.80 $\pm$ 0.10 <sup>ab</sup>	0.73 $\pm$ 1.10 <sup>ab</sup>	0.39 $\pm$ 0.84 <sup>b</sup>	1.09 $\pm$ 1.45 <sup>ab</sup>
Salt%	4.09 $\pm$ 0.69 <sup>bc</sup>	3.88 $\pm$ 0.98 <sup>c</sup>	6.58 $\pm$ 1.88 <sup>a</sup>	5.16 $\pm$ 1.49 <sup>b</sup>	5.30 $\pm$ 2.02 <sup>a</sup>	4.65 $\pm$ 1.54 <sup>b</sup>

<sup>a-d</sup>values within the same row with no common superscript differ significantly ( $p < 0.05$ ).



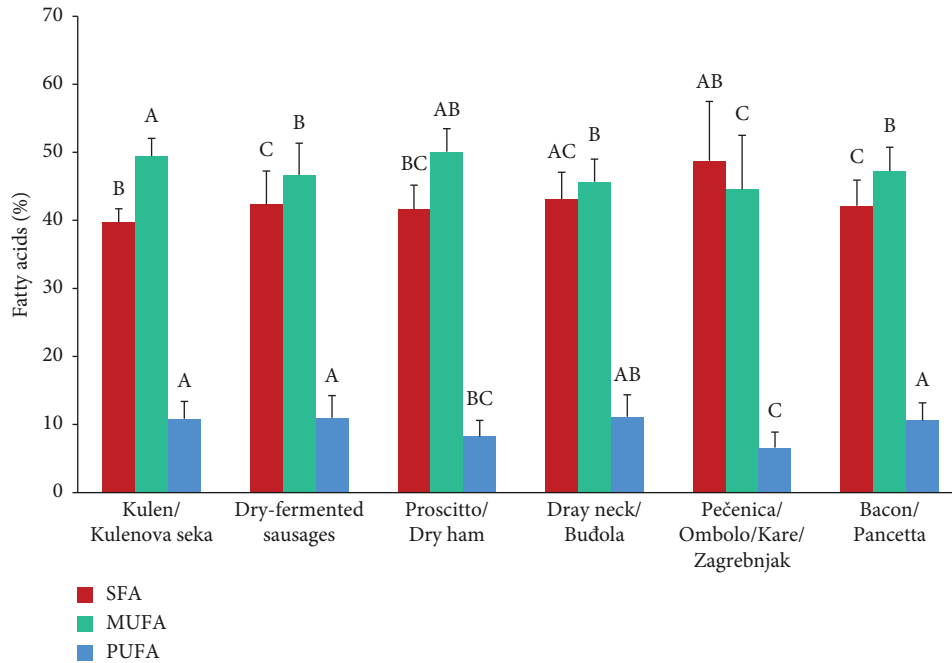


FIGURE 2: Percent-share (%) of fatty acids (mean  $\pm$  SD) across various TMPs. <sup>A-C</sup> values within a fatty acid group (SFA, MUFA, and PUFA) with no common superscript differ significantly ( $p < 0.05$ ).

fermented sausages ( $29.33 \pm 3.90\%$ ) > dry neck/*Budola* ( $27.88 \pm 3.85\%$ ) > bacon/pancetta ( $20.75 \pm 5.97\%$ ). When it comes to the coexistence of high protein and low-fat content, *Pečenica/Ombolo/Kare/Zagrebnjak* would be the TMP of choice. The carbohydrate content ranged from  $0.39 \pm 0.84\%$  (*Pečenica/Ombolo/Kare/Zagrebnjak*) to  $1.49 \pm 1.03\%$  (*Kulen/Kulenova seka*). These findings could be attributed to the production recipes that include the addition of sugars to accelerate fermentation [20]. The salt content varied from  $3.88 \pm 0.98\%$  in dry-fermented sausage to  $6.58 \pm 1.88\%$  in prosciutto/dry hams. Higher salt content values were determined by Pleadin et al. [36] in Istrian ( $5.51 \pm 0.96\%$ ) and Slavonian sausage ( $4.16 \pm 0.67\%$ ). The average amount of salt in meat filling used to stuff a sausage is 2.0–2.6%, but during ripening it increases up to 3.4–5.5% in the final product [47]. Laureati et al. [50] found the salt content to vary from 4.5–4.9% to 6.4–6.9% in Parmense and San Daniele to 8.3% in Tuscan hams. The reason behind the uneven salt content in prosciutto/dry hams, dry neck/*Budola*, *Pečenica/Ombolo/Kare/Zagrebnjak*, and bacon/pancetta lies in the curing techniques, which include surface salt rubbing and addition of unweighted salt amounts [12]. TMP saltiness depends on the amount of salt added and the duration of drying and ripening, affecting also sensory properties and microbiological stability of meat products.

**3.3. Mineral Content.** Minerals are nutrients that do not contain elemental carbon and are divided into macro- and microelements, depending on the amounts needed by the human body [51]. Meat and meat products are rich sources of minerals necessary for vital functions of the human body and have a better bioavailability as compared to the minerals

of plant origin. The proportion of minerals in meat depends on several factors, the most important being breeding practices, animal species, its age, gender, and feeding practices [52]. The results pertaining to the mineral composition of the TMPs under this study are presented in Table 4. The data revealed a statistically significant difference between mineral contents of different TMPs (Na ( $p < 0.001$ ), Ca ( $p < 0.001$ ), K ( $p = 0.029$ ), Mg (0.021), Cu (0.002), and Fe ( $p < 0.001$ )), the only exception being made by the Zn content ( $p = 0.167$ ).

The results showed the domination of sodium and potassium over other minerals. Salting of meat products, either during meat batter preparation or via surface rubbing, resulted in the supremacy of sodium content over the content of all other minerals. The salt content established in the studied products ranged from  $15.54 \pm 3.90$  g/kg in dry-fermented sausages to  $26.34 \pm 7.53$  g/kg in prosciutto/dry ham. Similar values were determined by Kos et al. [12] in the study conducted on Dalmatian/Istrian hams, where the average sodium content in Dalmatian and Istrian ham was 2,350.54 mg/100 g and 3,148.33 mg/100 g, respectively. Jiménez-Colmenero et al. [11] found lower Na values in dry-cured hams (1,200 mg/100 g), while Milešević et al. [53] determined similar sodium contents in dry-fermented sausages (1.24–1.80%), dry meat (1.04–2.56%), and bacon (0.80–2.04%).

These study results also show that, in addition to the high sodium content, TMPs also contain a high amount of potassium, which ranged from  $4.25 \pm 1.48$  g/kg in bacon/pancetta to  $6.25 \pm 5.93$  g/kg in dry-fermented sausages. High potassium content was also found in Istrian prosciutto [12], varying from 720.0 to 1,010.20 mg/100 g, while in Dalmatian prosciutto, lower values in the range of 366.0 to 538.80 mg/

TABLE 4: The results of mineral analysis (mean  $\pm$  SD) displayed based on the type of traditional meat product.

Parameter	<i>Kulen/Kulenova seka</i> (n = 34)	Dry-fermented sausages (n = 65)	Prosciutto/dry ham (n = 42)	Dry neck/ <i>Budola</i> (n = 22)	<i>Pečenica/Ombolo/Kare/Zarebnjak</i> (n = 29)	Bacon/pancetta (n = 50)
Na (g/kg)	16.37 $\pm$ 2.61 <sup>bc</sup>	15.54 $\pm$ 3.90 <sup>b</sup>	26.34 $\pm$ 7.53 <sup>a</sup>	21.86 $\pm$ 4.96 <sup>ac</sup>	21.20 $\pm$ 8.07 <sup>ac</sup>	18.61 $\pm$ 6.16 <sup>c</sup>
Ca (mg/kg)	326.94 $\pm$ 117.66 <sup>a</sup>	313.16 $\pm$ 73.17 <sup>a</sup>	328.83 $\pm$ 109.30 <sup>a</sup>	279.60 $\pm$ 142.42 <sup>ab</sup>	237.84 $\pm$ 49.37 <sup>b</sup>	224.11 $\pm$ 88.80 <sup>b</sup>
K (g/kg)	5.69 $\pm$ 1.14 <sup>a</sup>	6.25 $\pm$ 5.93 <sup>a</sup>	5.82 $\pm$ 1.47 <sup>a</sup>	5.26 $\pm$ 1.23 <sup>a</sup>	6.07 $\pm$ 1.60 <sup>a</sup>	4.25 $\pm$ 1.48 <sup>b</sup>
Mg (mg/kg)	343.31 $\pm$ 67.52 <sup>b</sup>	358.59 $\pm$ 93.96 <sup>b</sup>	362.27 $\pm$ 107.25 <sup>ab</sup>	362.48 $\pm$ 82.64 <sup>ab</sup>	465.91 $\pm$ 261.56 <sup>a</sup>	363.52 $\pm$ 162.88 <sup>ab</sup>
Cu (mg/kg)	1.73 $\pm$ 0.46 <sup>a</sup>	1.63 $\pm$ 0.56 <sup>a</sup>	1.49 $\pm$ 0.43 <sup>ab</sup>	1.47 $\pm$ 0.44 <sup>ab</sup>	1.18 $\pm$ 0.46 <sup>b</sup>	1.53 $\pm$ 0.67 <sup>ab</sup>
Zn (mg/kg)	40.84 $\pm$ 37.92	33.88 $\pm$ 7.14	43.75 $\pm$ 67.03	45.35 $\pm$ 24.55	27.68 $\pm$ 6.75	31.58 $\pm$ 18.89
Fe (mg/kg)	19.39 $\pm$ 5.94 <sup>a</sup>	18.40 $\pm$ 6.56 <sup>a</sup>	13.89 $\pm$ 3.64 <sup>b</sup>	17.69 $\pm$ 8.40 <sup>ab</sup>	13.77 $\pm$ 6.73 <sup>b</sup>	12.76 $\pm$ 3.76 <sup>b</sup>

<sup>a-c</sup>values within the same row with no common superscript differ significantly ( $p < 0.05$ ).

100 g were determined. In a Polish study, which analysed traditional and conventional pork hams, the potassium content of 2,475–3,493 mg/kg was determined. Wiejska sausages were also proven to represent a rich source of this mineral, whose average value in these sausages equalled to 3,150.04 mg/kg [2, 54, 55]. Marcos et al. [56] determined up to 5,800 mg/kg of potassium in various traditional Portuguese sausages, which is consistent with our results for *Kulen/Kulenova seka* (5,692.94 mg/kg).

Onwards, our results for calcium content in traditional sausages were in accordance with some earlier studies [54, 56], but values somewhat higher than those of Kos et al. [12] were obtained for prosciutto/dry hams, i.e., more similar to those of Jiménez-Colmenero et al. [11], who claimed the TMP calcium content to range from 12 to 35 mg/100 g. Meat products have been found to be an excellent source of iron, zinc, and magnesium [2, 11], that is, of micronutrients essential for vital functions and responsible for numerous health benefits [57]. Gonzalez-Tenorino et al. [57] determined higher levels of Zn and Fe in traditional sausages than in industrial ones. They concluded that the obtained results correspond to the age of an animal, but also to the migration of Fe ions into the meat from the equipment used on smaller-scale family farms. In this research, the levels of Zn varied from  $27.68 \pm 6.75$  mg/kg in *Pečenica/Ombolo/Kare/Zarebnjak* to  $45.35 \pm 24.55$  mg/kg in dry neck/*Budola*. In prosciutto/dry ham, the determined levels of Zn (43.75 mg/kg) were higher than those reported by other authors; for example, Lucarini et al. [58] stated that in Italian dry-cured hams, the levels of Zn vary from 20.8 to 27.2 mg/kg, while in traditional Polish smoked hams, Halagarda et al. [54] found the Zn content of 27.3 mg/kg.

Zinc content determined in dry-fermented sausages ( $33.88 \pm 7.14$  mg/kg) was similar to the previously published literature data. For instance, Djinovic-Stojanovic et al. [59] found 32.7 mg/kg of Zn in dry-cured sausages, while Marcos et al. [56] determined that in various traditional Portuguese sausages, the concentration of Zn spans from 5 to 43 mg/kg. According to Halagarda et al. [55], the amount of Zn contained by Wiejska sausage equals to 29.77 mg/kg. The highest Fe content was determined in *Kulen/Kulenova seka* (19.39 mg/kg), followed by dry-fermented sausages (18.40 mg/kg), dry neck/*Budola* (17.69 mg/kg), prosciutto/dry ham (13.89 mg/kg), *Pečenica/Ombolo/Kare/Zarebnjak* (13.77 mg/kg), and finally bacon/pancetta (12.76 mg/kg). The obtained results are in accordance with the previously published data on similar traditional products [11, 12, 54, 56, 58]. The lack of Fe in the body causes anaemia, which is a public health problem affecting a quarter of the world's population, so that it is important to point out that meat products are rich Fe sources [11]. The highest Mg concentration was determined in *Pečenica/Ombolo/Kare/Zarebnjak* (465.91 mg/kg), while the concentrations found in prosciutto/dry ham, dry neck/*Budola*, and bacon/pancetta were approximately the same (362.27 mg/kg, 362.48 mg/kg, and 363.52 mg/kg, respectively). The magnesium amounts established in dry-fermented sausages and *Kulen/Kulenova seka* were mutually similar, but lower than the above (358.59 mg/kg and 343.31 mg/kg, respectively). The results of

other authors in this regard also vary from 40 to 410 mg/kg in traditional Portuguese sausages [56], over 222 mg/kg in Serbian dry-fermented sausage and 289 mg/kg in their dried meat products [59], 174 mg/kg in smoked Polish ham [54], to 32.33 mg/kg in Dalmatian prosciutto [12]. Copper is also an essential but at the same time a toxic microelement, its action depending on the amounts introduced into the organism. The lack of it is associated with bone malformations and osteoporosis, altered immune response, and decreased Fe metabolism [60]. In this research, the lowest Cu concentration (1.18 mg/kg) was determined in *Pečenica/Ombolo/Kare/Zarebnjak*, while the highest concentration of 1.73 mg/kg was found in *Kulen/Kulenova seka*. Halagarda and Wójciak [2] stated that in traditional meat products, Cu concentration can significantly vary and range from 300 to even 2,360  $\mu$ g/kg.

**3.4. Amino Acid Composition.** Dry-cured and fermented meats represent a significant source of proteins of high biological value. Based on the latest investigations performed on selected meat products, it has been confirmed that most meat products have digestible indispensable amino acid scores (DIAAS) over 100 and that various processing methods will increase both standardized ileal digestibility (SID) of amino acids (AA) and DIAAS [61], even in cases where the meat is further processed prior to consumption. Dietary protein requirements aim at providing the minima of nine essential amino acids (EAAs) or indispensable amino acids (IAAs). These are necessary for new protein structures and cannot be produced by the human body in physiologically significant amounts, so that they must be supplied as crucial components of a balanced diet [62, 63]. EAAs include histidine, isoleucine, leucine, valine, lysine, threonine, phenylalanine, methionine, and tryptophan. Proteolysis that results in the break of peptides into free amino acids via endogenous and microbial enzymes contributes to the nascence of both volatile and nonvolatile flavours. The main amino acids derived from the muscle tissue decomposition that takes place in fermented sausages are alanine, leucine, isoleucine, and glutamic acid. Small amounts of branched-chain amino acids, particularly leucine, isoleucine, and valine, are metabolized, yielding the major aroma compounds that include aldehydes, branched alcohols, and methyl acids [64].

In this study, twenty-one FAAs were detected in traditional meat products (Table 5). Among them, alanine (136.4–505.40 mg/100 g), histidine (700.04–3,987.3 mg/100 g), isoleucine (253.63–1,072.61 mg/100 g), leucine (163.53–669.13 mg/100 g), and proline (382.30–2,421.87 mg/100 g) were present in high amounts in all samples, histidine, isoleucine, and leucine thereby being the essential amino acids. However, large variations in FAA content were observed in the same product type due to the differences in microbial microflora and physicochemical variables.

The highest alanine value was found in prosciutto/dry ham, followed by *Kulen/Kulenova seka*, but without statistical differences between the two. The reason behind this could be the high protein content associated with a longer

TABLE 5: Amino acid profile of various types of traditional meat products.

Parameter/mg/100 g	Kulen/Kulenova seka (n = 34)	Dry-fermented sausages (n = 65)	Prosciutto/dry ham (n = 42)	Dry neck/Budola (n = 22)	Pečenica/Ombolo/Kare/Zarebnjak (n = 29)	Bacon/pancetta (n = 50)
Alanine (ALA)	425.20 ± 147.58 <sup>a</sup>	176.78 ± 76.57 <sup>ab</sup>	505.40 ± 164.34 <sup>a</sup>	324.25 ± 38.93 <sup>ab</sup>	141.29 ± 124.75 <sup>b</sup>	136.4 ± 34.49 <sup>b</sup>
Arginine (ARG)	32.88 ± 40.63 <sup>ab</sup>	3.01 ± 3.98 <sup>ab</sup>	172.73 ± 96.80 <sup>a</sup>	145.74 ± 177.03 <sup>a</sup>	50.32 ± 48.70 <sup>ab</sup>	44.13 ± 12.36 <sup>a</sup>
Asparagine (ASN)	1.53 ± 1.63 <sup>b</sup>	0.13 ± 0.16 <sup>ab</sup>	0.58 ± 0.47 <sup>ab</sup>	1.01 ± 0.28 <sup>a</sup>	0.15 ± 0.26 <sup>b</sup>	0.36 ± 0.14 <sup>ab</sup>
Aspartic acid (ASP)	131.06 ± 55.50 <sup>ab</sup>	23.65 ± 17.95 <sup>b</sup>	223.20 ± 132.81 <sup>a</sup>	33.83 ± 29.38 <sup>b</sup>	33.40 ± 11.94 <sup>b</sup>	21.32 ± 13.67 <sup>b</sup>
Cysteine (CYS)	2.97 ± 0.62 <sup>b</sup>	2.57 ± 1.67 <sup>b</sup>	42.81 ± 31.89 <sup>a</sup>	35.02 ± 15.44 <sup>ab</sup>	19.59 ± 5.90 <sup>ab</sup>	7.05 ± 10.41 <sup>b</sup>
Glutamic acid (GLU)	206.77 ± 61.92 <sup>ac</sup>	57.93 ± 29.92 <sup>b</sup>	241.50 ± 74.45 <sup>a</sup>	119.57 ± 29.10 <sup>abc</sup>	43.26 ± 44.12 <sup>bc</sup>	42.21 ± 10.02 <sup>abc</sup>
Glutamine (GLN)	85.80 ± 25.69 <sup>b</sup>	24.04 ± 12.42 <sup>b</sup>	100.21 ± 30.89 <sup>b</sup>	49.61 ± 12.07 <sup>a</sup>	17.95 ± 18.31 <sup>b</sup>	17.51 ± 4.16 <sup>b</sup>
Glycine (GLY)	4.27 ± 2.49	2.43 ± 3.05	1.64 ± 1.65	3.91 ± 3.09	4.74 ± 2.09	3.31 ± 2.22
Histidine* (HIS)	2,505.44 ± 1185.11 <sup>bc</sup>	945.07 ± 349.77 <sup>a</sup>	3,987.34 ± 857.53 <sup>b</sup>	2,175.98 ± 258.10 <sup>bc</sup>	700.04 ± 857.25 <sup>c</sup>	822.98 ± 305.16 <sup>c</sup>
Hydroxyproline (HYP)	6.10 ± 2.08 <sup>a</sup>	5.80 ± 2.56 <sup>ab</sup>	7.29 ± 2.83 <sup>a</sup>	8.94 ± 2.64	2.92 ± 1.80 <sup>b</sup>	3.10 ± 1.07 <sup>b</sup>
Isoleucine* (ILE)	864.89 ± 321.78 <sup>a</sup>	253.63 ± 118.41 <sup>ab</sup>	1072.61 ± 261.23 <sup>a</sup>	536.45 ± 68.06 <sup>b</sup>	260.72 ± 233.80 <sup>b</sup>	266.35 ± 69.16 <sup>b</sup>
Leucine* (LEU)	509.86 ± 156.97 <sup>b</sup>	163.53 ± 77.51 <sup>a</sup>	669.13 ± 110.43 <sup>bc</sup>	384.98 ± 44.81 <sup>bc</sup>	201.30 ± 145.37 <sup>c</sup>	186.21 ± 46.00 <sup>c</sup>
Lysine* (LYS)	12.40 ± 5.28 <sup>b</sup>	3.65 ± 1.31 <sup>a</sup>	20.52 ± 2.32 <sup>b</sup>	11.13 ± 2.17 <sup>c</sup>	8.75 ± 1.07 <sup>bc</sup>	5.01 ± 1.49 <sup>c</sup>
Methionine* (MET)	16.75 ± 11.89 <sup>b</sup>	0.45 ± 0.67 <sup>ac</sup>	25.26 ± 26.31 <sup>b</sup>	6.67 ± 13.06 <sup>bc</sup>	1.28 ± 0.95 <sup>c</sup>	1.98 ± 0.82 <sup>abc</sup>
Phenylalanine* (PHE)	240.36 ± 82.81 <sup>a</sup>	71.19 ± 33.75 <sup>c</sup>	390.93 ± 58.67 <sup>ac</sup>	192.38 ± 39.53 <sup>b</sup>	142.92 ± 15.52 <sup>bc</sup>	90.23 ± 24.69 <sup>b</sup>
Proline (PRO)	1,730.39 ± 809.27 <sup>b</sup>	643.07 ± 307.63 <sup>a</sup>	2,421.87 ± 363.10 <sup>b</sup>	1,275.44 ± 270.23 <sup>b</sup>	382.30 ± 356.00 <sup>c</sup>	514.25 ± 177.22 <sup>c</sup>
Serine (SER)	11.47 ± 7.13 <sup>a</sup>	4.73 ± 2.84 <sup>ab</sup>	22.27 ± 16.39 <sup>ab</sup>	19.00 ± 1.99 <sup>b</sup>	4.26 ± 7.02 <sup>ab</sup>	7.20 ± 1.69 <sup>b</sup>
Threonine* (THR)	8.68 ± 5.88 <sup>b</sup>	2.67 ± 1.47 <sup>a</sup>	13.36 ± 7.03 <sup>b</sup>	6.68 ± 1.01 <sup>b</sup>	1.80 ± 2.83 <sup>a</sup>	3.39 ± 0.82 <sup>b</sup>
Tryptophan* (TRP)	9.01 ± 6.35 <sup>a</sup>	4.52 ± 2.90 <sup>a</sup>	42.14 ± 19.3 <sup>b</sup>	20.32 ± 10.38 <sup>b</sup>	12.30 ± 3.57 <sup>b</sup>	7.96 ± 4.84 <sup>b</sup>
Tyrosine (TYR)	22.56 ± 21.27 <sup>b</sup>	1.44 ± 1.95 <sup>a</sup>	108.87 ± 37.56 <sup>b</sup>	49.21 ± 28.00 <sup>b</sup>	47.17 ± 14.55 <sup>b</sup>	25.92 ± 8.20 <sup>b</sup>
Valine* (VAL)	105.11 ± 41.07 <sup>b</sup>	33.75 ± 15.38 <sup>a</sup>	131.25 ± 19.25 <sup>bc</sup>	68.13 ± 9.06 <sup>bc</sup>	44.67 ± 12.90 <sup>bc</sup>	30.53 ± 8.72 <sup>c</sup>

\*essential amino acids (EAAs). <sup>a-c</sup>values within the same row with no common superscript differ significantly ( $p < 0.05$ ).

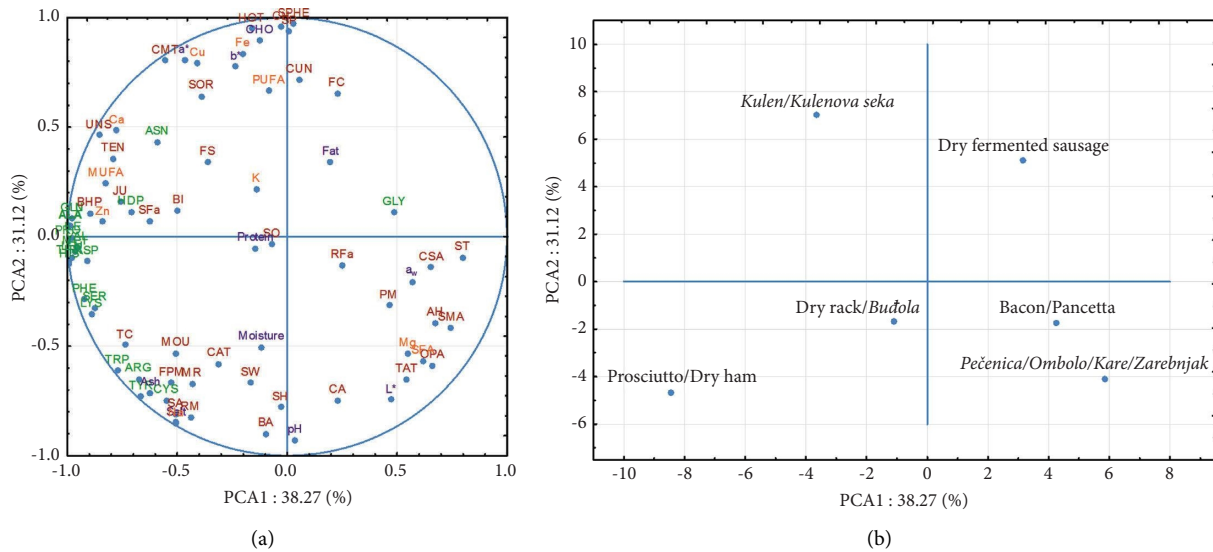


FIGURE 3: Loading plot of the variables encompassed (a) and score plot (b) obtained by the principal component analysis of sensory evaluation scores, physicochemical traits, fatty acids, amino acids, and mineral content of *Kulen/Kulenova seka*, dry-fermented sausages, Prosciutto/Dry ham, dry neck/*Budola*, dry loin/*Pečenica/Ombolo/Kare/Zarebnjak*, and bacon/pancetta.  $L^*$ , lightness;  $a^*$ , redness;  $b^*$ , yellowness; CMT, colour of the muscle tissue; CUN, colour uniformity; FC, fat content; CAT, colour of the adipose tissue; CH, cohesiveness; SH, surface humidity; MR, marbling; TC, tyrosine crystals; FS, favourable smell; UNS, unfavourable smell; SO, smoky odour; TEN, tenderness; JU, juiciness; SA, saltiness; SW, sweetness; SOR, sourness; BI, bitterness; SP, spiciness; HOT, hotness; BA, buttery aroma; AH, aromatic herbs; SPHE, spice herbs; RM, ripen meat; BHP, biochemical properties; FPM, fresh pork meat; MOU, moulds; CSA, cross-section attractiveness; SMA, smell attractiveness; ST, smell typicality; CA, consistency attractiveness; TAT, taste attractiveness; PM, product maturity; RfA, richness of favourable aromas; SFA, steadiness of favourable aromas; OPA, overall product attractiveness; ALA, alanine; ARG, arginine; ASN, asparagines; ASP, aspartic acid; CYS, cysteine; GLU, glutamic acid; GLN, glutamine; GLY, glycine; HIS, histidine; HYP, hydroxyproline; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; PHE, phenylalanine; PRO, proline; SER, serine; THR, threonine; TRP, tryptophan; TYR, tyrosine; VAL, valine; CHO, carbohydrates; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

drying/ripening. Consequently, alanine concentrations were significantly lower in other samples studied. A similar trend was observed for other FAAs mentioned above, out of which proline, the main component of collagen, was present in high amounts in all samples studied. The release of free amino acids is a result of the higher initial activity and higher levels of cathepsins, calpains, and aminopeptidases, as well as the action of microbial proteinases. Many factors can inhibit or reduce the activity of the above enzymes, including water activity and salinity [65]. The representation of FAAs correlated with the aroma of the product. Glutamic acid (42.21–241.50 mg/100 g), lysine (3.65–20.52 mg/100 g), and tryptophan (4.52–42.14 mg/100 g) were represented in lower amounts, alanine thereby making the exception. Furthermore, the reason for obtaining high values of SD in amino acid content lies in various technological processes of domestic producers from different regions. FAAs are formed by proteolysis, which can vary for the same type of TMPs and mainly depends on the fermentation conditions (time, protein content, microbiota, temperature, and relative humidity). Larger standard deviations indicate the variety of such products that can be found on the market but also indicates the need for production standardization.

The principal component analysis (PCA) was conducted to elaborate on the relationships between variables and provide a deeper description and understanding of the samples. The analysis included sensory evaluation scores, physicochemical traits, fatty acids, amino acids, and mineral

content. The first principal component (PCA1) explained 38.27% of the variation, and the second principal component (PCA2) explained 31.12% of the variation. Cumulatively, the first two principal components (PCs) explained 69.39% of the total variance.

The measurements and PCs presented in Figure 3 were interpreted based on the correlations between each variable and each principal component. Variables close to each other were considered positively correlated, those separated by  $180^\circ$  as negatively correlated, and those separated by  $90^\circ$  as independently correlated. It was found that smell typicality, cross-section attractiveness, water activity, and glycine content had the highest positive contribution to PCA1 characterization. Almost all free amino acids, biochemical properties, Zn content, juiciness, and MUFA content accounted for the negative side of PCA1. These relations emphasize the strong negative correlation between the water activity value and free amino acid content because amino acids exhibit an affinity for hydrogen bonding with water molecules, thereby reducing water activity [66]. Furthermore, strong positive relationships are shown between MUFA and biochemical properties, mainly described by the oxidation of fat, as concluded in previous research by Chizzolini et al. [67]. Previous research reported the antioxidative effect of Zn [68]. Therefore, positioning Zn close to variables related to oxidation was not expected. A possible explanation

could be related to the findings presented by Trzaskowski et al. [69], reporting that serine, cysteine, histidine, proline, and glutamate/aspartate have been found to form stable complexes with zinc.

The positive side of PCA2 is primarily correlated with the intensity of spicy herbs, hotness, spiciness, and consistency, as well as the content of carbohydrates, Fe content, and yellowness ( $b^*$ ). Spicier meat products in Croatia are characterized by the addition of hot red pepper, leading to an elevation of yellowness ( $b^*$ ). Furthermore, higher Fe content in dry meat products could be related to a faster drying rate, increasing the dry matter content and consequently the Fe content, as well as the product consistency. The most significant variables on the negative side of PCA2 are pH value, buttery aroma, surface humidity, colour of adipose tissue, and sweetness, suggesting a strong positive correlation between these variables. Previous research on traditional dry meat products reports that the main reactions responsible for aroma development are lipid degradation (oxidative reactions), microbial fermentation of carbohydrates, and enzymatic hydrolysis of proteins and lipids, generating free amino acids and fatty acids [70]. These reactions lead to the formation of aldehydes, ketones, esters, furans, lactones, and other compounds responsible for specific sensory stimuli. The longer the production process, the higher the degradation of proteins and lipids, leading to a richer aroma. Additionally, the pH value of TMPs increases during processing due to proteolysis and the formation of basic compounds [71]. Therefore, the formation of a favourable aroma and an increase in pH value are directly related to proteolysis and lipolysis products.

In the score plot on Figure 3(b), a clear separation is observed among *Kulen/Kulenova seka*, dry-fermented sausage, prosciutto/dry ham, and *Pečenica/Ombolo/Kare/Zarebnjak*. Bacon/pancetta is positioned next to *Pečenica/Ombolo/Kare/Zarebnjak*, while dry neck/*Buđola* is at the centre of the coordinate system. As seen in Figure 3(a), overall product attractiveness (OPA) is placed in the quadrant between positive side of the PCA1 and negative side of the PCA2. When excluding sensory traits, higher OPA values are associated with products featuring elevated water activity and pH values, as well as increased lightness ( $L^*$ ), SFA, and Mg content. Comparing Figures 3(a) and 3(b), *Pečenica/Ombolo/Kare/Zarebnjak* is closest to OPA, along with bacon/pancetta. Similarly, prosciutto/dry ham is more characterized by free amino acid content, saltiness, and ripened meat aroma due to the much longer production period when those traits could develop more. *Kulen/Kulenova seka* is characterized by redness ( $a^*$ ), yellowness ( $b^*$ ), sourness, and a favourable smell related to the addition of a higher amount of spices. In a similar manner, dry-fermented sausage is more characterized by fat content analysed chemically and sensorially. In conclusion, the analysis of various traditional meat products (TMPs) in Croatia reveals distinct clusters in the score plot, indicating significant differences in composition and characteristics.

#### 4. Conclusions

The Croatian traditional meat products show high quality, as evidenced by their nutritional composition, characterized by

high protein content, a well-balanced amino acid profile, and richness in essential biogenic minerals vital for body functions. This nutritional profile holds appeal for health-conscious consumers worldwide seeking high-quality protein sources and natural mineral supplements. Despite having elevated levels of saturated fatty acids and salt, this study highlights the nutritional composition of TMPs in line with health recommendations, potentially influencing consumer perceptions and purchasing decisions, particularly among those seeking healthier alternatives to conventional processed meats. Furthermore, the sensory analysis reveals that all analysed samples exhibit good acceptability, indicating alignment with consumer taste preferences. This suggests potential appeal to consumers globally, particularly in markets where traditional, artisanal food products are valued.

Given the statistically significant differences in physicochemical properties, amino acid, fatty acid, and mineral composition, as well as sensory characteristics, it is evident that TMPs vary greatly due to different recipes and production technologies employed by rural households. Similar variations are observed across different Croatian production regions, indicating opportunities for standardization and improvement. Future research could focus on identifying optimal production techniques and ingredient combinations to enhance the nutritional profile, sensory properties, and overall quality of TMPs, while investigating consumer preferences, purchasing behaviour, and willingness to pay.

Considering the results obtained and the general belief among consumers that traditional meat products are healthier than their conventional counterparts, coupled with strategic promotion efforts, it is expected that nutritionally valuable and high-quality Croatian TMPs will gain recognition and appreciation in the global market. Overall, this research offers valuable insights into the quality parameters and appealing sensory attributes of Croatian TMPs, laying the groundwork for future studies aimed at optimizing production practices, promoting consumer health, preserving cultural heritage, and capitalizing on market opportunities.

#### Data Availability

Research data used to support this study are not shared.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this study.

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