PAPER

QUALITY OF ISTRIAN AND SLAVONIAN DRY-FERMENTED SAUSAGES

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ABSTRACT

The aim of this study was to investigate nutritive and sensorial quality of Istrian and Slavonian pork-meat dry-fermented sausages. Sensorial analysis resulted in statistically significant differences ($p<0.05$) between these products in 11 sensorial parameters analysed. Significantly higher protein content was determined in Istrian in comparison with Slavonian dry-fermented sausage, although the latter content significantly varied across the producing households. No significant differences in particular fatty acid esters and saturated, monounsaturated and polyunsaturated fatty acids were obtained. Although some of the nutritional quality indices failed to meet health recommendations, the obtained values are consistent with data published for pork meat products.

Keywords: Croatian households, dry-fermented sausages, nutritive properties, sensory properties, traditional pork meat sausages

1. INTRODUCTION

A long tradition of dry-cured meat production in rural households exists all over the world, especially in the Mediterranean countries. A very important group of such products are dry-fermented sausages, whose properties widely vary due to various processing procedures often lacking uniformity (TALON *et al.,* 2007; GARCÍA-GONZÁLEZ *et al*., 2013). Suitable chemical and sensory markers enable better linkage between raw matter and processing parameters, and thus result in higher uniformity and consistency of the products (CHIZZOLINI *et al*., 1996; VIRGILI and SCHIVAZAPPA, 2002; ZANARDI *et al*., 2010). A wide variety of dry-fermented sausages is characterized by specific flavour investigated into by a number of studies focused on clarifying the control mechanisms affecting the flavour development (TOLDRÁ, 1998; GARCÍA-GONZÁLEZ *et al*., 2013).

The highest impact on sensory properties of these products is that of smoking and ripening operations (JERKOVIĆ *et al*., 2010; KOVAČEVIĆ, 2014). Of particular significance are the processes of fat lipolysis, free fatty acids' formation, and degradation and oxidation of short-chain fatty acids, since these key reactions, taking place during ripening, affect the formation of specific odour and taste of the final product (KOZAČINSKI *et al*., 2006; MILIČEVIĆ *et al*., 2014; MARUŠIĆ *et al*., 2014). Raw material quality is influenced by farm animals' genotype, manners of their keeping and feeding, procedures applied before slaughtering, and post-slaughtering conditions. Technological processes, such as conditioning, fermentation, drying, smoking and ripening, as well as different technological parameters, such as temperature, relative humidity and air/smoke velocity, influence the properties of the final product, too. Due to the application of various technological processes, the activity of technological microflora, especially during the fermentation process and long-term maturation of sausage stuffing during production, complex microbiological, physicochemical and biochemical changes take place in fundamental building materials (fats, proteins and carbohydrates), resulting in water loss and increase in dry matter weight (KOVAČEVIĆ, 2014).

During the recent decades, studies devoted to fermented meat products have mainly focused on evaluation of physicochemical, microbiological and sensory properties (COMI *et al*., 2005, RANTSIOU *et al*., 2005, DI CAGNO *et al*., 2008) in order to contribute to the better characterization of final products, the definition of unique quality markers and the improvement of product specification protocols essential when dealing with products of the protected designation of origin (PDO) or geographical indication (PGI) (BOGDANOVIĆ *et al*., 2016). However, it is known that significant differences exist among traditional dry-fermented sausages produced in different countries or even in the same country (KOS *et al*., 2009; ZANARDI *et al*., 2010). As for traditional dry-fermented sausages produced in Croatia, Slavonian sausage is produced in the eastern Croatia and represents a very important Croatian PGI food brand. The same applies to Istrian sausage produced in the western Croatia, which is in the process of becoming a protected product. Even though both sausages are produced from pork meat, Istrian sausages contain less fat tissue and are spiced with pepper and garlic. The ingredient that makes these sausages specific is the local wine (Malvasia). Unlike these, Slavonian sausages are spiced with garlic and red pepper and contain in general a larger amount of fat tissue.

The production of Slavonian sausage calls for the minimum of 70% of the second- and the third-category pork meat and the minimum of 30% of solid fat tissue, while the production of Istrian sausage makes use of the meat of the same categories, but of not more than 6% of bacon. Mincing (6-8 mm hole for Slavonian and at least 10 mm for Istrian sausage) is followed by the addition of salt and spices; in case of Slavonian sausage, sweet and hot red pepper and garlic are added, while in case of Istrian sausage pepper and Malvasia enriched with garlic extract and prepared according to a specific procedure, are added. In both cases, a thoroughly mixed mixture of the above-detailed ingredients is stuffed in pork small intestine used as casing, which should be at least 70 cm long when it comes to Slavonian, and not longer than 50 cm when it comes to Istrian sausage. In the further course of Slavonian sausage production, the product is conditioned for a day, cold-smoked for 14 days tops, and ripened and dried in chambers for at least 45 days at temperatures not higher than 16°C, relative humidity thereby being kept at 70-85%. Since Istrian sausages should not be smoked, the subsequent course of their production upon stuffing involves ripening in chambers at temperatures of 9-16°C and with relative humidity of 65 to 85%. Istrian sausages can be released to the market 30 days after the production commencement, while the entire production of Slavonian sausages takes 60 days at least. As for their physicochemical properties, Slavonian sausage is allowed to contain no more than 40% of fat, while water activity should be kept below 0.90; on the other hand, Istrian sausage should contain 16% of proteins at the minimum and 40% of water at the maximum.

According to specification, Slavonian sausage has an elongated cylinder shape; one sausage pair should be at least 35 cm long and measure 2-3 cm in cross-section (MA, 2019). The casing is reddish/brown and should be free of smudges, folders, cracklings and surface moulds. The texture is expected to be solid and elastic, but not rubberish; the sausage should be easy-to-cut, not prone to crumbling, and easy-to-chew. The stuffing should be dark red in its cross-section, except for the fat tissue, which is coloured either white or orange; the stuffing resembles a mosaic, should be well entangled and free of holes, lacking a dark margin under the casing. Prior to cutting, Slavonian sausage has a smoky smell of an ash tree, hornbeam or beech; once the sausage is cut, the smell of fermented meat and garlic is released. Istrian sausage assumes the shape of an elongated cylinder, measuring at least 50 cm in length and having a rounded diameter. The casing should not be damaged and should tightly adhere to the stuffing. In its cross-section, the sausage is solid, mosaic-like, the meat thereby being coloured red and the fat tissue being coloured white. The sausage should be compact and holes-free, while its stuffing should not get separated during cutting.

Given the fact that among complex factors that drive consumer dry-fermented sausages' choice, nutritive and sensory properties are the major criteria, the aim of this study was to investigate into these qualities of the most important types of traditional Croatian dry-fermented sausages. The study was performed on Istrian and Slavonian sausages produced in different households situated in two Croatian main fermented meat production regions. Based on fatty acid content, nutritional quality indices were also assessed for both types of products.

2. MATERIALS AND METHODS

2.1. Sampling

The study involved 40 samples of traditional Croatian dry-fermented sausages named Istrian (n=20) and Slavonian sausage (n=20). Sausages were collected randomly during a two-year period (2018-2019) from markets, fairs and producing households. The products originated from two Croatian regions: the western region (Istra and Primorje) and the eastern region (Slavonia and Baranja). Pursuant to the Ordinance on Meat Products (OG 62/18) adopted by the Republic of Croatia, these sausages belong to the group of dry-cured sausages falling into the category of non-thermally processed meat products. The sausages were produced according to traditional recipes using traditional technologies observed by the producing rural household. To the end of their production, pork meat of the first, the second and the third category was used, together with fat and various amounts of salt and spices, depending on the sausage type (KOVAČEVIĆ, 2017).

2.2. Sensory characteristics

Sensory analysis was carried out by a trained panel (of 9 assessors). The assessors were selected and generically trained according to the ISO standard (ISO 11132:2012). Sensory analysis was carried out in the Sensory Laboratory of the Faculty of Food Technology and Biotechnology according to ISO 8589:2007. Sensory assessment made use of a quantitative descriptive analysis (QDA) and a unipolar numerical intensity scale was developed in collaboration with the Centro Studi Assaggiatori (Brescia, Italy). The intensity of each sensorial property was assessed using an ascending left-to-right numerical scale, "zero" thereby representing the absence of a given sensorial property and "nine" its highest intensity. At each panel session, a repeated sample of a given product group was available; for each assessor, a number of statistical parameters descriptive of his/her efficacy was calculated.

Plates containing single-coded samples were served into sensory compartments, together with all materials required for sensory assessment. Each assessor carried out a sensory assessment of the intensity of objective visual food item properties (colour of the minced meat, colour uniformity, fat content, cohesiveness), smell-related properties (favourable smell, unfavourable smell, smoky smell), taste-related properties (tenderness, juiciness, saltiness, sweetness, sourness, bitterness, spiciness) and aroma (coming from aromatic herbs, spice herbs, ripe meat, biochemical properties, fresh pork meat, moulds). The above was followed by the assessment of subjective properties (cross-section attractiveness, smell attractiveness, consistency attractiveness, maturity, richness of appealing aromas, steadiness of appealing aromas, overall attractiveness), which made use of the same numerical, intensity-measuring scale.

2.3. Physicochemical analysis

Samples were cut into small pieces and homogenized for 15 sec at 6,000 rpm using a Grindomix GM 200 (Retsch, Haam, Germany). Sample preparation was completed in full line with ISO 3100-1:1991. All samples were analysed for physicochemical parameters within the next 48 hours upon arrival into the Laboratory. The extracted fat was stored in a refrigerator at -18°C pending fatty acid composition analysis carried out within the next 48 hours. All chemicals used for analyses were of an analytical grade.

The pH value was determined in a homogenate diluted with distilled water (1:10, p/v) using a pH/Ion 510 – Bench pH/Ion/mV Meter (Eutech Instruments Pte Ltd/ Oakton Instruments, USA) according to the pH/Ion 510 Instruction Manual. Water activity (a_w) was measured at the room temperature (20±2°C) using a Rotronic Hygrolab 3 (Rotronic AG, Bassersdorf, Switzerland). The pH-value and water activity given for each sample represent the mean value of two independent measurements. The water content was determined gravimetrically (ISO 1442:1997) at 103°C in an oven (UF75 Plus, Memmert, Schwabach, Germany), while the ash content was established according to ISO 936:1998 by

virtue of burning the samples in a furnace at 550°C (LV9/11/P320 Nobertherm, Lilienthal, Germany). The total fat content was determined using the Soxhlet method (ISO 1443:1973), which involves digestion of a sample in an acidic environment followed by fat extraction with petroleum ether using a Soxtherm 2000 automated device (Gerhardt, Munich, Germany). The total protein content was determined using the Kjeldahl method (ISO 937:1978) that employed a Unit 8 Basic digestion block (Foss, Höganäs, Sweden) and an automated distillation & titration device (Vapodest 50s, Gerhardt, Munich, Germany). The salt content determination made use of the multiple standard addition potentiometric technique that employs an ion-selective electrode and a Na EasyPlusTM analyser (Mettler Toledo, Germany). Based on the established sodium content, the representation of sodium chloride (salt) was determined stoichiometrically. The carbohydrate content was calculated based on the water, ash, total protein and fat content.
Each sausage sample was analysed in duplicate and the results were expressed in form of weight percentage (%) with the accuracy of 0.01%. Quality control of analytical methods used was performed using the Reference Material (RM) TET003RM (Fapas, York, England).

2.4. Fatty acid profile

Sample preparation for the analysis of fatty acid methyl esters was described earlier by PLEADIN *et al.* (2019). Fatty acid methyl esters were analysed using gas chromatography (GC) according to ISO 12966-4:2015 and EN ISO 12966-4:2015. To the above effect, a 7890BA gas chromatographer equipped with flame ionization detector (FID), a 60-m DB-23 capillary column having an internal capillary diameter of 0.25 mm and the stationary phase thickness of 0.25 μm (Agilent Technologies, Santa Clara, USA) was used. The components were detected by FID at the temperature of 280°C, hydrogen flow rate of 40 mL/min, air flow rate of 450 mL/min and nitrogen flow rate of 25 mL/min. The initial column temperature was 130°C; after a minute, it was increased by 6.5°C/min until the temperature of 170°C was reached. The temperature was further increased by 2.75°C/min until the temperature of 215°C was attained. The latter temperature was maintained for 12 min and then further increased rate by 40°C/min until the final column temperature of 230°C was reached, the latter being maintained for 3 min. One mL of a sample was injected into a split-splitless injector at the temperature of 270°C and with the partition coefficient of 1:50. The carrier gas was helium (99.9999%), flowing at the constant rate of 43 cm/sec. Fatty acid methyl esters were identified by comparing their retention times with those of fatty acid methyl esters contained by the standard mixture.
The results were expressed as a percent-share (%) of an individual fatty acid in total fatty acids, the accuracy thereby being 0.01%. Each sausage sample was analysed in duplicate. The material used for quality control was CRM BCR 163 (Institute for Reference Materials and Measurements, Geel, Belgium) that has a specified content of seven individual fatty acids.

2.5. Nutritional quality of lipids

Data on fatty acid composition in terms of the mean values obtained by the analysis of two replicates, were used for the calculation of the following lipid quality indices: the atherogenic index (AI), the thrombogenic index (TI) and the hypocholesterolaemic/hypercholesterolaemic ratio (HH). The atherogenic index (AI) indicates the relationship between the sum of the main saturates and the sum of the main non-saturates. This parameter was calculated as follows:

$$AI = [(C12{:}0 + (4 \times C14{:}0) + C16{:}0)] / [\sum MUFA + PUFA\ n\text{-}6 + PUFA\ n\text{-}3]$$

(ULBRITCTH and SOUTHGATE, 1991)

The thrombogenic index (TI) is defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic FAs (MUFA, PUFA n-6 & PUFA n-3). The index was calculated as follows:

$$TI = (C14{:}0 + C16{:}0 + C18{:}0) / [0.5 \times \sum MUFA + 0.5 \times PUFA\ n\text{-}6 + 3 \times PUFA\ n\text{-}3) + + (PUFA\ n\text{-}3/PUFA\ n\text{-}6)].$$

The ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids (HH) takes into account well-known effects of certain fatty acids on cholesterol metabolism (SANTOS-SILVA *et al*., 2002). It was calculated as follows:

$$HH = (C18{:}1n\text{-}9 + C18{:}2n\text{-}6 + C20{:}4n\text{-}6 + C18{:}3n\text{-}3 + C20{:}5n\text{-}3 + C22{:}5n\text{-}3 + C22{:}6n\text{-}3) / (C14{:}0 + C16{:}0)$$

(ULBRITCTH and SOUTHGATE, 1991).

2.6. Data analysis

Statistical analysis was performed using the SPSS Statistics Software 22.0 (SPSS Statistics, NY IBM, 2013) and the Big Sensory Soft (Centro Studi Assaggiatori, Brescia, Italy, 2005). In order to determine the differences between Istrian and Slavonian dry-fermented sausage in terms of physicochemical properties, fatty acid composition and sensory parameters, the independent sample t-test was used. The decisions on statistical significance were made at the significance level of $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Sensory characteristics

Sensory qualities of fermented meat products are adjudicated based on their aroma, appearance, flavour, texture, aftertaste and sound properties (FLORES, 2011). It is important to point out that contrary to some foods, sensory assessment of fermented meat products, including fermented sausages, is not standardized, since no consensus on sensory attributes that should be evaluated hasn't been reached yet. In several papers, these attributes have been selected and assessed through complex procedures involving sensory vocabulary generation so as to be able to compile a lexicon to be used to describe a sensory profile of a given fermented meat product, as well as through quantitative-descriptive analysis (RUIZ-PÉREZ-CACHO *et al*., 2005; GARCÍA-GONZÁLEZ *et al*., 2006; GARCÍA-GONZÁLEZ *et al*., 2008; BENEDINI *et al*., 2012). As for Croatian household-produced dry-fermented sausages, it has already been concluded that variations in their overall quality, especially sensory characteristics, represent a huge problem (FRECE *et al*., 2014).

The results of sensory evaluation of both Istrian and Slavonian dry-fermented sausage using quantitative descriptive analysis, carried out within this study frame, are shown in Fig. 1.

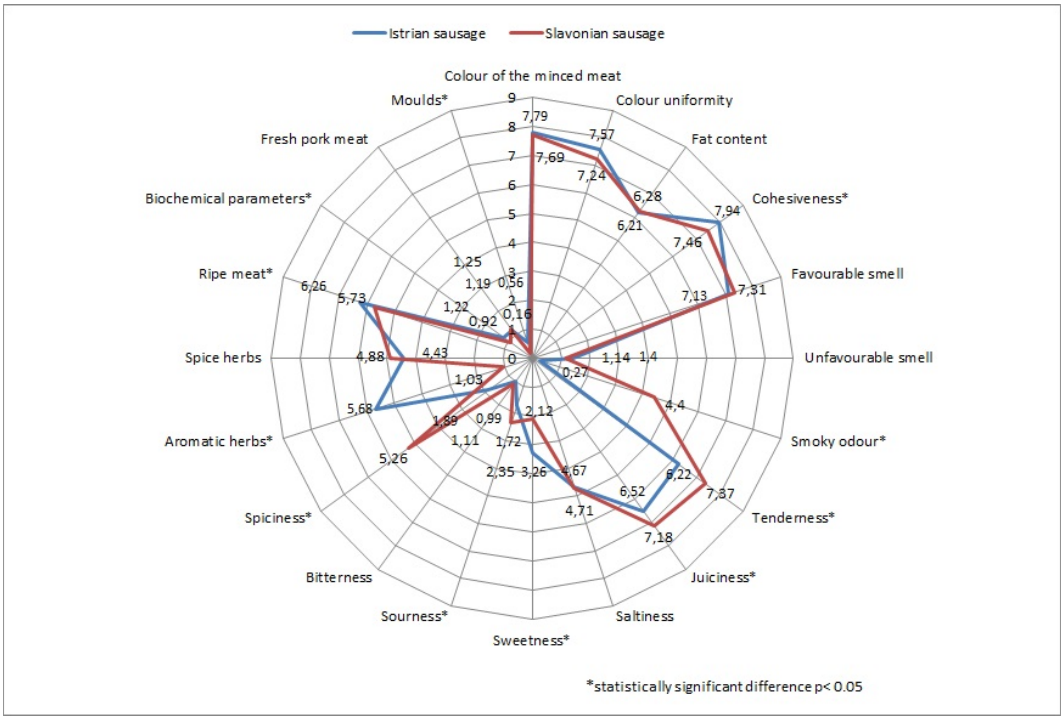

Figure 1. Descriptive sensory profile of objective characteristics (appearance, odour, texture, taste and aroma) of Istrian and Slavonian dry-fermented sausage.

Sensorial analysis resulted in statistically significant differences ($p<0.05$) between these products in 11 out of the total of 20 sensory characteristics analysed, including cohesiveness ($p=0.001$), smoky odour ($p<0.001$), tenderness ($p<0.001$), juiciness ($p=0.008$), sweetness ($p<0.001$), sourness ($p<0.001$), spiciness ($p<0.001$), aroma generated by the presence of aromatic herbs ($p<0.001$), ripe meat quality ($p=0.011$), biochemical parameters ($p=0.016$) and mould presence ($p<0.001$). These significant differences can be attributed to the differences in processing and to the use of different ingredients and spices during the production of the two.

The cohesiveness is stronger in Istrian in comparison with Slavonian sausage. Slavonian sausage scored higher for its smoky odour than did Istrian sausage, whose score in this regard was around 0. The latter was to be expected given that Slavonian sausage is smoked with cold smoke, while Istrian sausage should not be smoked at all. Slavonian sausage scored higher for tenderness and juiciness. It is known that these parameters are in correlation with moisture and fat content. Istrian sausage was shown to be sweeter, as oppose to Slavonian sausage, which was proven sourer, but they turned out to be equally salty. Istrian sausage must have a delicate taste (never sour) and should be moderately salty, while the taste of Slavonian dry-fermented sausage, according to its specifications (MA, 2019), should be mildly hot but never bitter, coming as a result of the combination of fermented meat, garlic, salt and red pepper used in its production. Slavonian dry-fermented sausage is spicier as compared to Istrian one due to the presence of red pepper spiking, while Istrian sausage scores higher when it comes to aromatic herbs, which can also be attributed to spices added, i.e. garlic and local wine Malvasia.

The principal intensity scales applied in sensory profiling of similar dry-fermented sausages varied in terms of the different point intensity scales as well as the unstructured line scale (FLORES, 2011). The lack of consensus regarding the application of sensory scales sometimes makes the comparison and discussion of the results of similar studies quite difficult. However, the results pertaining to the texture descriptors (tenderness, juiciness), obtained in this study, are in agreement with those reported by KOS *et al.* (2015) for dry sausages made from domestic pig and wild boar meat. In general, studies that employed sensorial analysis in order to compare dry-ripened sausages with similar fat levels reported higher sensory scores for texture attributes (FONSECA *et al.*, 2015). Moreover, the amount of fat affects the colour of smoked sausages and is responsible for a high score obtained on the sensory attributes' assessment scale (AHMAD and AMER, 2012), as was the case in this study, too. The smell and the taste of smoked sausages come as the result of decomposition of carbohydrates, lipids and proteins mediated by enzymes, as well as the due to the spices used in the production and the production process itself (KABAN and KAYA, 2009). The results descriptive of smell- and taste-related attributes of Istrian and Slavonian sausages, obtained in this study, can also be compared to those obtained by KOS *et al.* (2015), who also claimed that dry sausages made from domestic pig and wild boar meat have an intense aroma, higher salinity, more pronounced spiciness, pronounced herbal and ripe meat aroma, and more stable flavour.

The intensity of subjective characteristics of Istrian and Slavonian dry-fermented sausage, assessed as a part of descriptive sensory analysis, is shown in Fig. 2.

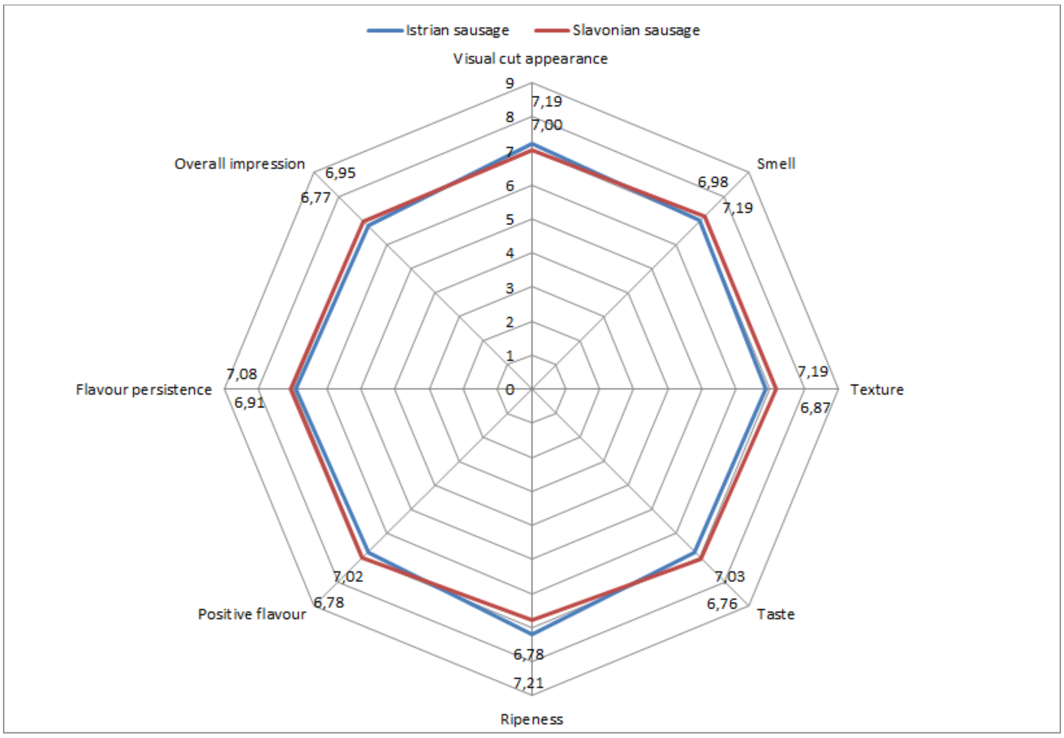

Figure 2. Descriptive sensory profile of subjective characteristics of Istrian and Slavonian dry-fermented sausage

No significant differences (p>0.05) in any of the assessed attributes were established, showing a good acceptability of both types of dry-fermented sausages.

3.2. Physicochemical properties

Unlike industrial production, household technologies applied during the production of fermented sausages are not regulated, so that a number of production-related factors, such as uneven weight and quality of raw materials and differences in production technologies, result in the diversity of composition of the finished products. Therefore, physicochemical properties of dry-fermented sausages show huge variability across individual producers and production periods (KOZAČINSKI *et al*., 2008). Industrial production of traditional dry sausages leans on traditional recipes also used in rural households, but, as opposed to seasonal household production, is carried out under controlled processing conditions and is not seasonal in nature, thus allowing for a continuous market supply throughout a year. Recent studies have shown that, due to healthy trends in meat products' consumption in terms of low-fat and low-salt products' preference, meat producers are facing a new challenge that comes down to attaining fat and salt reduction without any loss in sensory qualities (JIMÉNEZ-COLMENERO *et al*., 2009, FLORES *et al*., 2013).
Physicochemical parameters of Istrian and Slavonian dry-fermented sausage determined in this study are shown in Table 1. The results pertinent to chemical parameters indicate a similar nutritional composition of these two products, with statistically significant differences ($p<0.05$) in pH value, protein and ash content.

Table 1. Physicochemical parameters of Istrian and Slavonian dry-fermented sausages.

Parameter	pH	Water (% w/w)	Protein (% w/w)	Fat (% w/w)	Ash (% w/w)	Salt (% w/w)	CH (% w/w)
Istrian dry-fermented Sausage	5.72±0.59	24.99±5.79	30.66±5.05	38.76±8.44	5.00±0.72	5.51±0.96	0.59±0.43
Slavonian dry-fermented Sausage	5.12±0.27	28.10±4.28	26.68±3.57	39.48±8.58	4.54±0.59	4.16±0.67	1.20±0.92
p value	0.000	0.085	0.013	0.801	0.049	0.234	0.490

CH - carbohydrates.
Results are expressed as the mean value (%, mean±SD); one sausage sample was taken and analysed in duplicate.

The pH value is an indicator of fermentation and ripening of a meat product (SALGADO *et al*., 2005) and is commonly used for the assessment of their shelf-life. In dry-fermented sausages, the pH value spans from 4.7 to 6.3 (DELLAGLIO *et al*., 1996; ZANARDI *et al*., 2010; DEMEYER *et al*., 2000; MORETTI *et al*., 2012; PLEADIN *et al*., 2014). In this study, the mean pH values equalled to 5.11 for Slavonian sausage and 5.72 for Istrian sausage, i.e. were within the acceptable range specified above.
Literature data suggest that, due to the longer drying and ripening of dry-fermented sausages and a high share of lean meat used in the preparation of their stuffing, water and protein content in finished ripened sausages are on an equal level (about 30-40% w/w),

suggesting a high nutritional value of the finished product (PLEADIN *et al*., 2014). In dry-fermented sausages, the water content mostly raises up to 40% w/w (VIGNOLO *et al*., 2010). The ratio of water over proteins of 1.2 to 1.3 is typical of dry sausages (INCZE, 2007). In this study, a higher protein as compared to water content was determined in Istrian dry-fermented sausage, while in Slavonian dry-fermented sausage the ratio of water over proteins was determined to be 1.05. When analysing dry sausages produced in Croatian households, water over protein ratio ranged from 1.0 to 2.1, showing that these products are of a high nutritional value (KOZAČINSKI *et al*., 2008).
The amount of total fat present in dry-fermented sausages generally varies widely (21.70 to 55.40% w/w) depending on the recipe and the producing household, but also on the origin of raw materials. Such variations can be attributed to the differences in the amount of added fatback and the choice of more or less fatty meat made by individual manufacturers (PLEADIN *et al*., 2014). The ash content of meat products generally ranges from 3.52% to 6.06% w/w (OCKERMAN and BASU, 2007; JIMÉNEZ-COLMENERO *et al*., 2010; KAROLYI and ČURIĆ, 2012).
Salt (sodium chloride) is an essential ingredient of fermented sausages, so that meat products are one of the richest sodium chloride food sources contributing to the increased water and fat binding capacity, the formation of colour, taste and texture of the product and its microbiological safety. The salinity of a product depends on the amount of added salt and the duration of drying and ripening of the product (WIRTH, 1986), and has a significant impact on hardness and elasticity of meat products and their resistance to chewing (KOVAČEVIĆ *et al*., 2010). In fermented sausages' stuffing, an average share of salt ranges from 2.0% to 2.6%, whereas during the drying process the value keeps growing up to its final level found in the finished product (OCKERMAN and BASU, 2007; STAHNKE and TJENER, 2007). JIMÉNEZ-COLMENERO *et al*. (2001) demonstrated that larger amounts of salt exceeding the value of 4-6% have been established in numerous fermented meats studies, which was also the case in both sausages under this study (4.16-5.51%).
Since the glucose content in meat is too low or much too variable, different carbohydrates like glucose, sucrose, lactose, maltodextrin, corn syrups, starches and sorbitol are added into fermented sausages' stuffing (MASTANJEVIĆ *et al*., 2017) so as to enhance the growth of technological microflora (LUCKE, 1994). Sugars, mostly glucose, facilitate dry sausage fermentation process, since they pose as a substrate for the lactic acid production and contribute to the specific aroma development. Sugars added into fermented sausage stuffing in the maximal percent-share of 2% (usually 0.3-0.8% w/w) ensure the pH decrease from the initial 5.8-6.0 down to 4.8-5.4 (LUCKE, 2000). The carbohydrate content established in sausages under this study (0.59% in Istrian and 1.20% in Slavonian dry-fermented sausage) can be explained in view of the above.

3.3. Fatty acid profile

Table 2 shows the fatty acid composition and fat quality indices of Istrian and Slavonian dry-fermented sausage. Statistical analysis of the fatty acid composition-related data did not show any significant differences between these two types of sausages ($p>0.05$).
No significant differences in fatty acid esters and saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were obtained, revealing these sausages to have the fatty acid profile of a typical pork meat product, with small composition variations attributable to the differences in the amount of added fatback and the stuffing fatness. The proportions of fatty acid groups found in both analysed sausages were as

follows (in descending order): MUFA (46.66-46.83%) > SFA (42.91-43.00%) > PUFA (10.26-10.33%). This trend has also been confirmed in earlier studies of fatty acid profile of meat and meat products (MARUŠIĆ RADOVČIĆ *et al.*, 2014; WOODS and FEARON, 2009; PLEADIN *et al.*, 2014).

Table 2. Fatty acid composition (% of total fatty acids) of Istrian and Slavonian dry-fermented sausage.

Fatty acid	Istrian dry-fermented sausage	Slavonian dry-fermented sausage	p value
C10:0	0.09±0.01	0.09±0.01	0.565
C12:0	0.08±0.01	0.08±0.01	0.968
C14:0	1.41±0.11	1.43±0.15	0.714
C16:0	25.39±0.30	25.92±1.22	0.274
C17:0	0.39±0.09	0.40±0.12	0.779
C18:0	14.31±1.12	14.21±1.13	0.817
C20:0	0.43±0.23	0.43±0.19	0.961
C21:0	0.46±0.18	0.44±0.10	0.805
SFA	**42.91±1.98**	**43.00±2.22**	**0.921**
C16:1	2.31±0.34	2.56±0.29	0.087
C18:1*t*	0.19±0.04	0.20±0.03	0.749
C18:1*c*	44.18±2.50	43.74 ±2.99	0.689
C22:1	0.14±0.09	0.17±0.07	0.386
MUFA	**46.83±2.65**	**46.66±3.13**	**0.885**
C18:2n-6	8.97±3.17	9.13±2.64	0.586
C18:3n-6	0.23±0.02	0.24±0.03	0.469
C18:3n-3	1.01±0.16	0.95±0.20	0.365
PUFA	**10.26±3.25**	**10.33±2.59**	**0.805**
n-6	9.22±3.19	9.37±2.64	0.902
n-3	1.04±0.15	0.96±0.20	0.246

Results are expressed as the mean value (%, mean±SD); one sausage sample was taken and analysed in duplicate.
LOD (limit of detection)=0.05%.
SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids.

As proven earlier, fatty acids most represented in different pork meat sausages are oleic (18:1n-9c), palmitic (16:0), stearic (18:0) and linoleic (C18:2n-6c) acid (LEŠIĆ *et al.*, 2017). The same was established in the study of fatty acid composition of industrial Slavonian Kulen (PLEADIN *et al.*, 2014). Oleic acid, as the predominant fatty acid in meat and meat products, ranged from 43.74±2.99% in Slavonian sausage to the similar 44.18±2.50% in Istrian dry-fermented sausage, and accounts for roughly 94% of all MUFAs in both products. Among SFAs, palmitic and stearic acid were shown to be dominating and accounted for roughly 93% of all SFAs in both types of sausages. Linoleic acid, as the dominating PUFA, accounted for roughly 87-88% of all PUFAs in both sausages.

3.4. Nutritional quality indices

PUFA/SFA and n-6/n-3 ratios are the most common parameters used to evaluate the nutritional quality of fat (JIMENEZ-COLMENERO *et al.*, 2010). Additional indices, which take into account different effects that a single fatty acid might have on the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation, i.e. the atherogenic (AI) and the thrombogenic index (TI), were also calculated. In order to gain insight into the effect of fatty acids on blood cholesterol, the ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids (H/H) was determined, too.
Nutritional fat quality indices determined in this study for Istrian and Slavonian dry-fermented sausage are shown in Table 3. No statistically significant differences ($p>0.05$) in any of the indices were determined between the two.

Table 3. Nutritional fat quality indices calculated for Istrian and Slavonian dry-fermented sausage.

Nutritional quality indices	Recommended value	Istrian dry-fermented sausage	Slavonian dry-fermented sausage	p value
n-6/n-3	< 4	8.90±3.07	10.28±4.18	0.340
PUFA/SFA	< 0.4	0.24±0.08	0.24±0.06	0.971
AI	< 1	0.55±0.05	0.56±0.05	0.791
TI	< 1	1.45±0.13	1.46±0.13	0.879
H/H	as higher	2.01±0.17	1.98±0.17	0.676

H/H - hypo-/hypercholesterolaemic fatty acids ratio; AI - atherogenic index; TI - thrombogenic index.
Results are expressed as the mean value (%, mean±SD); one sausage sample was taken and analysed in duplicate.

Literature has revealed that consumption of animal fats is related to an excessive intake of SFAs and an increased proportion of n-6 PUFAs (n-6/n-3 ratio). It has been shown that in the current diet of consumers from western countries, the n-6/n-3 ratio is roughly 15-20+, while according to health recommendations it should be less than 4 if the incidence of chronic diet-related diseases is to be reduced (SIMOPOULOS, 2002; CORDAIN *et al.*, 2005). In order to meet health recommendations or reduce the risk of cardiovascular, autoimmune and other chronic diseases, the PUFA/SFA ratio should be higher than 0.4 (SIMOPOULOS, 2002). In this study, the PUFA/SFA ratio was in line with health recommendations, whereas n-6/n-3 ratio was approximately two to three times higher than the maximal recommended value. The mean n-6/n-3 ratios obtained in this study for Istrian and Slavonian sausage are not extremely high (about 10), however, still not in accordance with health recommendations; at least, they are consistent with the results of previous studies of similar pork meat products (LEŠIĆ *et al.*, 2017).
The AI is considered to be a particularly useful index because, in addition to describing MUFA content, it places the emphasis on myristic acid (C14: 0), which is believed to have the most harmful cardiovascular effects (HIGGS, 2002). The AI index takes into account the fact that some saturates, primarily myristic and palmitic acid, are considered to be pro-atherogenic (since they facilitate the adhesion of lipids onto the cells the immune and the circulatory system are composed of), while non-saturates are considered to be anti-atherogenic (since they inhibit the formation of plaques and diminish the levels of

esterified fatty acids, cholesterol and phospholipids, therefore preventing micro- and macro-coronary disease) (ULBRITCTH and SOUTHGATE, 1991). It is assumed that AIs below 1 are beneficial to human health (PLEADIN *et al*., 2019). For both types of sausages under this study, the AIs having the mean values of about 0.55 were in line with the recommendation, and similar to those found in other studies of different types of sausages (DEL NOBILE *et al*., 2009; STAJIĆ *et al*., 2011; RAZMAITE and ŠVIRMICKAS, 2012; ROMERO *et al*., 2013).

The TI indicates the risk of blood clotting and represents the ratio of pro-thrombogenic (certain saturated) and anti-thrombogenic (unsaturated) fatty acids. For both sausages analysed within this study, the TIs were about 1.5 times higher than the recommended values, which is consistent with literature data on similar types of sausages (DEL NOBILE *et al*., 2009; ROMERO *et al*., 2013). Recent studies show that fatty acids with an even number of C atoms (lauric, myristic and palmitic acid) increase the concentration of total and LDL cholesterol, as well as promote not only coagulation, but also inflammatory processes and insulin resistance (CALDER, 2015).

The H/H index takes into account known effects of certain fatty acids (especially oleic and linoleic acid) involved in cholesterol metabolism. The higher value of this index shows better effects for human health (SANTOS-SILVA *et al*., 2002). Oleic acid, cis-MUFA fatty acids in general and linoleic acid can reduce both total and LDL cholesterol, thereby reducing the risk of cardiovascular disease (CALDER, 2015). Due to the wide range of potential beneficial biological effects (cell membrane functionality, gene expression and lipid metabolism), n-3 PUFAs play a role in the prevention and treatment of inflammatory processes, thus reducing the risk of cardiovascular disease and some cancers (ARTERBURN *et al*., 2006). In this study, the H/H values approximated to 2 for both types of sausages; such a value is typical of meat, especially pork meat (SANTOS-SILVA *et al*., 2002; RAZMAITE and ŠVIRMICKAS, 2012).

4. CONCLUSIONS

Despite of the fact that Istrian and Slavonian dry-fermented sausages are produced in two climatically different regions, they are nutritionally related. Significant differences exist in their sensory properties due to the differences in traditional recipes used in their production. However, from the sensory evaluation standpoint, the existing sensory characteristics are highly accepted by consumers. Since this research showed that certain nutritional indices, especially n-6 over n-3 ratio, are not within the desirable limits and in line with health recommendations, modifications to the fatty acid composition of these sausages, to be attained primarily through changes in animal feeding practices, are needed in order to improve their nutritional properties and ultimately their beneficial effects on consumer health.

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