TOWARDS REPRODUCIBILITY OF TRADITIONAL FERMENTED SAUSAGES: TEXTURE PROFILE ANALYSES AND MODELLING

Article Highlights

- Texture profiles of three traditional dry-fermented sausages were investigated
- Regression analyses were used to correlate chemical composition and texture characteristics
- Differences were significant between sausages of the same type produced in different facilities
- Regression analyses showed that moisture content was the most effective for texture profile
- PCA revealed the best reproducibility of analysed characteristics for Petrovská klobása

Abstract

The aim of this study was to investigate textural characteristics of three traditional dry fermented sausages (Sremski kulen, Lemeški kulen and Petrovská klobása) manufactured in different small-scale facilities in northern Serbia, and to correlate them with physicochemical and sensory characteristics. The sample sausages were supplied by different local traditional producers. The textural characteristics were correlated with physicochemical and sensory characteristics using multiple linear regression analysis and principal component analysis. Differences in physicochemical characteristics reflected even more notable differences in texture characteristics. Regarding regression equations, obtained results showed that moisture content was significant for hardness, springiness and cohesiveness. Hardness was also influenced by fat content, while chewiness was influenced by protein content. Principal component analysis separated samples of Petrovská klobása, as the group with the most reproducible analysed characteristics. Obtained results of statistical analyses should provide knowledge for possible improvements of the traditional production, in a way that these sausages could be produced in different facilities with consistent textural characteristics.

Keywords: dry fermented sausage, MLR, PCA, texture analysis.
granted a protected designation of origin (PDO) label, according to Serbian legislation [5,6]. They are handcrafted, usually in small-scale facilities during winter, when atmospheric temperatures are around 0 °C or lower. In the traditional production of these sausages, curing salts and starter cultures are not used, and the required fermenting microorganisms originate from the meat itself or from the environment. Sausage batter is stuffed in pig appendix (Sremski kulen, Lemeški kulen) or pig large intestines (Petrovská klobása), and undergoes slow drying and ripening processes in traditional smoking/drying rooms [6-8].

Traditional practices in the small scale facilities lead to great variability in products’ properties (heterogeneous quality), as there is no strict uniformity in the product manufacturing by different homemade processors [1,3,9]. The texture is one of the most important components of meat products’ quality. Many factors affect the final texture of fermented sausages, including ingredients used, processing parameters, acidification method, drying/ripening conditions, as well as interactions among these factors over an extended period of time [10-13]. As it is one of the most important components of the sausage quality, it is important to create a product of textural attributes accepted by the consumer, but it is also important to ensure the product's quality reproducibility, i.e., a low variability of product characteristics [14]. However, insufficient work has been conducted so far to assess the reproducibility of the texture quality of traditional dry fermented sausages [15].

Multiple linear regression (MLR) is a mathematical tool that generates an equation to describe the statistical relationship between a dependent variable and one or more independent variables, and could be used to predict the textural parameters using the physicochemical parameters as independent variables. Also, evaluation of products’ quality variability requires a collection of numerous different data [14]. Using principal component analysis (PCA), the total number of variables could be reduced, and original variables could be transformed into new factors or principal components [4].

Considering the high interest of consumers for traditional dry fermented sausages and the lack of information about the texture characteristics of these products, which could contribute to their characterization, the aims of this work were:
  - to determine and compare physicochemical, sensory and textural characteristics of three traditional dry fermented sausage types (Sremski kulen, Lemeški kulen and Petrovská klobása) produced in different small scale facilities;
  - to explore the correlation of texture with physicochemical and sensory characteristics;
  - to obtain a deeper insight into the intercorrelation of investigated characteristics in order to understand factors leading to quality reproducibility.

EXPERIMENTAL

Samples

Three Serbian traditional dry fermented sausage types were considered in this study: Sremski kulen - S (samples S1-S4), Lemeški kulen - L (samples L1-L6), and Petrovská klobása - P (samples P1-P7). The manufacturing procedures for all three types of sausages are shown in Figure 1. The sausages of Sremski kulen and Lemeški kulen have a weight of 700-800 g and the diameter varies from 80 to 95 mm, whereas the weight of Petrovská klobása sausages is 500-600 g and the diameter ranges from 40 to 50 mm. The sample sausages were supplied in triplicate (different batches) by different local traditional producers and were stored at 4 °C before textural testing and sensory evaluation.

Figure 1. Manufacturing procedures of three Serbian traditional dry fermented sausages: S - Sremski kulen; L - Lemeški kulen; P - Petrovská klobása.

Chemical composition analyses

The pH was measured using the portable pH meter Testo 205 (Testo AG, USA) equipped with a combined penetration tip with temperature probe [16]. The measurements were performed by direct penetration in different areas of the internal part of the sausage. The pH meter was calibrated before and during
the readings using standard phosphate buffers (pH value of calibration buffers was 7.00 and 4.01 at 25 °C. Measurements were performed in triplicate. Sausage samples were homogenized (Waring 8010ES Blender, USA; capacity 1 L, speed 18000 rpm, duration of homogenization 10 s, temperature after homogenization <10 °C), packaged in polyethylene bags and stored at -20 °C until the determination of proximate chemical composition. Moisture content was determined by drying at 130 °C [17]; nitrogen concentration (N) was determined by the Kjeldahl method; protein content was calculated as total N×6.25 [18] and fat content was obtained by Soxhlet extraction using petroleum ether [19]. All analyses were performed in duplicate.

Texture profile analysis

Texture profile analysis (TPA) was performed as described by Bourne [20], at room temperature, using TA.HDplus texture analyser (Stable Micro Systems, Godalming, UK) equipped with a standard cylindrical plate of 75 mm in diameter. The samples (cylinders) 2 cm thick and 2.54 cm in diameter, after discarding the external layer of the sausage, were compressed twice to 50% of their original thickness at a constant test speed of 1 mm/s. The following parameters were determined: hardness (kg), springiness, cohesiveness and chewiness (kg). Hardness was defined by peak force during the first compression cycle. Springiness was defined as the rate at which a deformed sample goes back to its undeformed condition after the deforming force is removed. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Finally, chewiness was obtained by multiplying hardness, cohesiveness and springiness.

Colour measurements

Colour measurements were performed on the fresh cut of the sausage at room temperature using MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan); D-65 lighting, 2° standard observer angle; 8-mm aperture in the measuring head. Before each set of measurements, the instrument was calibrated using a white ceramic tile (CR-A43). Colour measurements were always performed in the center of the sausage sample. Sausage colour characteristics were expressed by CIE L*a*b* system (L* - lightness, a* - redness, b* - yellowness) [21].

Sensory evaluation

The sensory analyses were performed by the 9-member panel previously trained in descriptive analysis for different meat products [22]. Panel tasters were asked to score samples by using a 1-9 scale for each attribute to be evaluated, where 1 = very low and 9 = very high intensity. The descriptors considered chewiness, cohesiveness, and juiciness. For the assessment casing was removed and the sausages were cut into slices of approximately 3 mm thickness and served at room temperature on white plastic dishes. Water and unsalted toasts were provided to cleanse the palate between samples. Assessments were performed under natural light.

Statistical analysis

The statistical analysis was carried out using software packages Statistica version 12 and Minitab version 17. The analyses were conducted across all sausages types and the differences in investigated characteristics were analysed using ANOVA. Multiple linear regression models were fitted to estimate textural parameters such as hardness, springiness, cohesiveness and chewiness using physicochemical parameters (moisture, protein, and fat content, as well as pH) as independent variables. The best regression model for each dependent variable was based on the value of the coefficient of determination (R2), F-value and p-value. Coefficient of determination is a measure of the extent to which the total variation of the dependent variable is explained by the regression which means how close the data are to the fitted regression line. The F-value and the p-value are used to decide whether the model as a whole has the statistically significant predictive capability (at the 95% confidence level, the upper critical values of the F-distribution were given in the Statistica Handbook (NIST/SEMATECH e-Handbook of Statistical Methods)). Significance of each coefficient in the model was determined using a t-test, at the 5% significance level. The larger the magnitude of the t-value the more significant is the corresponding coefficient. PCA calculation was performed to obtain an insight into the relationships of data obtained by TPA test, physicochemical and sensory analysis and to reduce the number of relevant variables.

RESULTS AND DISCUSSION

The results of physicochemical, textural and sensory analyses of all three groups of traditional dry fermented sausages with basic statistics for characterization of the variability of the analysed samples, as well as ANOVA results, are given in Table 1.

Physicochemical characteristics

Based on the average moisture content, all three groups of samples were within the recommended values...
Table 1. Textural, physicochemical and sensory characteristics of traditional dry fermented sausages; HR - hardness [kg]; SP - springiness; CH - cohesiveness; CW - chewiness [kg]; W - water content (g/100 g); P - protein content (g/100 g); PH - pH value; D - diameter (mm); Chew - chewiness; Juic - juiciness; Cohe - cohesiveness; $L^*$ - lightness; $a^*$ - redness, $b^*$ - yellowness; SD - standard deviation; * a–c: means within the same column with different superscript letters are different ($p < 0.05$)

Table 1 data here...

for dry fermented sausages [23], i.e., lower than 35%. Sausages from group P had, on average, the lowest ($p < 0.05$) moisture content (21.56%). Consequently, their average fat content was the highest (37.91%), followed by L and S sausages, with significant differences between all of them. The lower moisture content in P sausages could be due to the difference in diameter since ripening processes were in approximately the same environmental conditions for the same period of time. Determined fat contents were in accordance with the fact that higher fat content is a dominant characteristic of dry fermented sausages [15,24,25]. Fat contributes to the sensory characteristics but also has a technological function in the manufacture of dry fermented sausages as it helps the continuous release of moisture from the inner layer of the sausage, a process necessary for undisturbed fermentation and flavour development [24]. Average protein contents for all three groups of samples were within the recommended values for dry fermented sausages, i.e., minimally 24% [23]. Protein content of S sausages (40.42%) was higher ($p < 0.05$) than that of P sausages (32.39%). Further, samples of P group showed the lowest ($p > 0.05$) pH value, with an average value of 5.36, whereas S and L sausages had average pH value of 5.69 and 5.60, respectively. These values are in accordance with the previous results for naturally fermented dry sausages [6,7,26], as well as with the fact that these sausages are generally characterised by low acidity [15]. Low process (environmental) temperatures limit the intensity of fermentation, and thus the pH does not decrease by more than 0.2-0.4 units, as it was previously found [27-29]. During the drying and maturation phases, the

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pH may return to similar values to those of the ripened meat due to the liberation of peptides, amino acids and ammonia from proteolytic reactions [29].

**Textural characteristics**

The highest mean hardness value was determined for sausages S (14.45 kg), followed by L (10.63 kg) and P (7.86 kg) sausages. The differences among mean hardness values were not significant (p > 0.05), probably because of high variability among measurements within each sausage type, with almost the same coefficients of variability for all three experimental groups. Hardness values obtained in the present study for P sausages, were in accordance with results reported for sucuk [30], while the ones obtained for S and L sausages were higher than those for sucuk [30], chorizo de Pamplona [25], and Italian low-acid sausage [31]. Cohesiveness mean values, ranging from 0.32 to 0.44 did not differ significantly among samples. According to Spaziani *et al.* [31], the lack of variability in cohesiveness could be a consequence of pH value being close to or over the isoelectric point. Springiness was the highest (p < 0.05) for S (0.53) sausages followed by L (0.45) and P (0.39) sausages, which did not differ significantly. Chewiness value had the same trend as hardness and springiness, being highest (p < 0.05) for S (3.17 kg) sausages (3.17 kg), and followed by L (1.81 kg) and P (0.96 kg) sausages, without significant difference between the last two. The major changes in texture take place in the fermentation process when the pH declines and the solubilized miofibrillar proteins aggregate to form a gel, thus transforming the meat mixture into a ripened sausage. In addition to fermentation, drying is an important factor affecting texture properties [11,30,31].

**Colour and sensory characteristics**

The quality of meat and meat products is often estimated based on colour and appearance [30]. Average $L^*$ values of analysed three groups of sausages were in the range of 32.06-33.04, with no significant differences (p > 0.05) between them. Regarding $a^*$ and $b^*$ values S sausages were significantly the lowest (p > 0.05), while L and P did not differ significantly. Colour characteristics values were lower than those reported for chorizo de Pamplona [25] but higher than the ones reported for sucuk [30]. Results of sensory analyses revealed no significant differences among analysed groups of sausage samples for juiciness and cohesiveness, while chewiness was found to be the lowest (p < 0.05) for L sausages.

**Multiple linear regression**

In order to better understand the effect of physicochemical characteristics (independent variables) on the textural parameter of sausages (dependent variables), a series of MLR analyses were performed. Firstly, models for each of the four textural outcome variables included a linear combination of all physicochemical predictor variables. Considering the obtained results (not reported), it was observed that regression models for springiness and chewiness were statistically significant (significant overall F-statistics), although each individual coefficient in the model was not (not-significant t-tests for the individual coefficients). This often indicates possible multicollinearity among the predictor variables [32]. Multicollinearity occurs when the model includes multiple factors that are correlated not just to the response variable, but also to each other.

The variance inflation factor (VIF), measures how much the variance of the estimated regression coefficient is “inflated” by the existence of correlation among the predictor variables in the model, and help to detect multicollinearity. The literature data indicates VIF exceeding 10 is a sign of serious multicollinearity requiring correction [33]. Multicollinearity does not affect the goodness of fit and the goodness of prediction, but it can be a problem if the purpose of the study is to estimate the contributions of individual predictors. For example, the VIF values were 28.04, 13.78, 49.78 and 1.60 for moisture, protein and fat content and pH value, respectively, and indicate a serious multicollinearity problem. In order to overcome this issue, a stepwise regression was performed. Results of stepwise MLR analysis, using Minitab v17, are given in Table 2. Regarding the regression equation for hardness, $R^2$ indicated that the model explained 87.4% of the variability, and both predictors in the model (moisture and fat content) are statistically significant according to their $t$-values. Also, a negative correlation between dependent and independent variables (negative values of regression coefficients) was observed, meaning that hardness decreases when moisture and fat contents increase. Olivares *et al.* [24], analysing texture parameters based on fat content, found a significant increase in hardness due to fat reduction only at longer ripening times due to the loss of moisture. Contrary to our findings, authors [11,25] found a significant correlation between hardness and pH value. Additionally, the significant positive correlation between protein content and hardness was found (Pearson $r = 0.86$), but this predictor was omitted from
Comparing the four models obtained by MLR, it could be concluded that some textural characteristics, namely hardness and chewiness, were more influenced by physicochemical characteristics than others. This implies that special attention should be paid to raw materials used in the production. Particularly, the fat content should be tightly controlled, because it was significantly correlated with all analysed textural characteristics either by MLR or Pearson correlation.

**Principal component analysis**

For a global view, the principal component analysis was performed using the data of instrumental (textural and colour measurements), physicochemical, and sensory characteristics of the fermented sausages samples (Figures 2 and 3).

Three main components (F1, F2 and F3) were used, which accounted for 73.77% of the total variance data, and the individual contributions of the components were 45.90, 15.87 and 12.00%, respectively. The fact that three dimensions were used, and explained only 73.77%, suggested the wide variability of the sample regarding the analysed parameters what was linked to different raw material/formulation and processing parameters. First PC (F1) was associated with most of the physicochemical parameters and redness. The second PC (F2) was mostly loaded by sensory and colour parameters, and the third one was mostly associated with textural characteristics.

Through first two components, it was only possible to separate samples of P sausages, which were allocated on the negative quadrant of F1, highly influenced by the fat content of the samples, and negatively correlated with moisture content and diameter size. As it was previously shown these samples had the highest fat content, with the lowest coefficient of variation. Data for other samples were scattered mostly in the positive quadrant of F1.
Regarding instrumental colour characteristics, F2 cooperated with separating sample L2, which had highest $L^*$, $a^*$ and $b^*$ values, comparing with all other samples, i.e., it was the lightest and with the highest share of red colour. Lightness ($L^*$) seems to be the most informative parameter for colour changes, but the importance of red ($a^*$) should not be ignored [25].

Further, the third PC (F3) was associated with hardness and protein content what was important in the characterisation of S3, S4 and L4 samples, since these samples were the highest in these characteristics compared to the others. Also, springiness and cohesiveness position on the positive quadrant of F3 was important for the characterisation of samples S1, S2, L5 and L6 being the highest in these values compared to other samples, and also being the highest in moisture content which was positively correlated with these two textural parameters. Analysed sensory properties of sausages (chewiness, cohesiveness and juiciness) did not make any difference for the characterisation of samples, and no significant correlations ($p > 0.05$, data not shown) were determined between instrumentally and sensory determined texture parameters.

The results of PCA lead to the following conclusions. Firstly, it could be observed that Petrovská klobása sausages are clearly distinct from the other two types of sausages. Secondly, all three types of sausages exhibited high variation which is evident from the cluster sizes (Figure 3). Considering the analysed parameters, a grouping of likewise variables into factors was evident, to some extent, although with several exceptions. More comprehensive and informative results regarding the relationship between the type of sausage and their quality characteristics could be obtained by using canonical correlation analysis (CCA), however, due to the very limited sample size, it was not possible to perform this type of analysis in this study.
CONCLUSION

Although the recipes and procedures in the traditional production of Sremski kulen, Lemeški kulen and Petrovská klobása are well known, obtained results showed differences in physicochemical and sensory characteristics even between sausages of the same type produced in different small scale facilities. These differences reflected to even more notable differences in texture characteristics. Obtained results of statistical analyses should provide knowledge for possible improvements of the traditional production, in a way that these sausages could be produced in different facilities with consistent textural characteristics.

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POSTIZANJE REPRODUCIBILNOSTI U PROIZVODNJI TRADICIONALNIH FERMENTISANIH KOBASICA: ANALIZA TEKSTURE I MODELOVANJE


Ključne reči: fermentisane suve kobasice, MLR, PCA, analiza teksture.