

## The effects of functional additives on quality characteristics of cooked sausages-mathematical approach

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### Abstract

*Individual quality parameters of cooked pork sausages were determined to examine the effect of various functional additives ( $\omega$ -3 fatty acids, natural additives, tocopherols, phytosterols) on some quality parameters (texture, color, sensory quality) of the final product. The analysis of variance and post-hoc Tukey's HSD test at 95% confidence limit showed significant differences between different samples. Low coefficients of variation were obtained for each applied assay, what confirmed the high accuracy of measurements. Principal Component Analysis (PCA) and Cluster Analysis (CA) were used for assessing the effect of different additives to cooked sausages. Using coupled PCA and CA of observed samples, the possible directions for improving the quality of product can be realized. Score analysis as a useful tool for accessing the effect of process parameters to cooked sausages confirmed that better results were obtained with  $\omega$ -3 fatty acids addition compared to samples with natural additives, and samples with tocopherol or phytosterol addition.*

**Keywords:** cooked sausages, functional additives, PCA, Cluster Analysis

### 1. Introduction

Natural food additives have been gaining more interest both from the public and food manufacturers, since consumers are increasingly aware of the toxicological implications of artificial additives. Generally, the consumers will choose a food with no additives, but if these are not available, the same consumer will choose, if possible, a food containing natural additives over synthetic ones (DE ALMEIDA & al. [1], CAROCHO & al. [2]). With this considerably increased demand for products with healthier characteristics there is a growing interest in the meat industry to replace artificial additives with natural compound (DE ALMEIDA & al. [1]). Natural additives are believed to be healthy and are of good quality (EYILER & OZTAN [3]). Also, modern consumers require nutritional food with functional properties (POLAK & al. [4]).

Recommendations about lipid consumption (WHO [5]) encourage to diminish the intake of saturated fat and to increase the unsaturated lipids. Meat products are recognized as one of the main sources of saturated fat, and the changes in its fat content and fatty acid (FA) profile could help to increase its nutritional quality (FERNANDÉZ-GINÉS & al. [6], ŠOJIĆ & al. [7]). The increasing demands in recent years for meat products with much lower fat content or healthier fatty acid composition drove to new recommending guidelines with reduced saturated fat intake and consumers' desire to lose weight (AKESOWAN [8],

ARCHER & al. [9]). The manufacturer's strategies to improve these products can be: the substitution of red meat by skinless poultry meat (ANDRÉS & al. [10]) the substitution of saturated fat with vegetable oils KOUTSOPOULOS & al. [11], OSPINA & al. [12]) and the use of fat replacers, such as guar gum, carrageenan, xanthan gum, and inulin (AKESOWAN [8], ANDRÉS & al. [10], ANDRÉS & al. [13, 14]). The increase in the consumption of n-3 polyunsaturated fatty acids (n-3 PUFA:  $\alpha$ -linolenic acid, ALA, eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) had been related to several studies with potential health effects as keep a normal cholesterol blood level (European Commission [15]). Recently, there has been a growing interest in developing products with a health concept such as a high  $\omega$ -3 fatty acid products (LEE & al. [16]). A certain way to increase the intake of n-3 PUFA is by incorporating them in traditional products.

The antioxidant potential of many natural compounds has been reported. Among these compounds, tocopherols, ascorbic acid, some phenolic compounds and carotenoids have been the most evaluated in food matrices, including meat products (MERCADANTE & al. [17]).

Phytosterols/stanols are used as a novel food ingredient with plasma cholesterol-lowering activity (PENNISI FORELL & al. [18]).

The aim of this research was to examine quality parameters of cooked sausages in the type of hot-dog, and to study the effect of various functional additives ( $\omega$ -3 fatty acids, natural additives, tocopherols, phytosterols) on the quality of the final product. Pattern recognition techniques (Principal Component Analysis - PCA and Cluster Analysis - CA) were applied to the experimental data (used as descriptors) to characterize and differentiate among the observed samples. In order to enable more comprehensive comparison between examined samples, standard score (SS), assigning equal weight to all assays applied, has been introduced. Principal component analysis (PCA), used as a pattern recognition technique, has been applied within assay descriptors to characterize and differentiate various analyzed samples.

## 2. Materials and Methods

The cooked sausage samples were manufactured at the factory for meat production and processing, "Topola" from Backa Topola, Serbia. Tests were performed on cooked sausages that are commercially produced (control) and sausages produced with functional additives (test samples). Further, tests were conducted with both cold (C) and hot (T) sausages, considering samples before and after heat treatment prior to consumption, respectively. Hot sausages were boiled in water at a temperature of 80°C for duration of 5 minutes, which resulted in an internal temperature of 72°C.

During the research used samples were coded as follows: 1- control, 2- with  $\omega$ -3 fatty acids addition (0.62%), 3- with natural additives addition, 4- with tocopherol addition (0.03%) and 5- with phytosterol addition (2.6%). The main mixture for sausage preparation consisted of: pork meat II category (30%), Hamburg bacon (30%), unclassified beef meat (15%), and water / ice (25%). Spices and additives for control sample and samples 2, 4 and 5 were: the nitrite salts (2.0%), potato starch (2.0%), emulsifiers (E407 and E471) (1.8%), Na lactate (E325) (1.0%), spices (0.64%), di, tri, and polyphosphates (E450, E451 and E452) (0.17%), enzymes (0.15%), Na-ascorbate (E301) (0.13%), color (E120) (0.06%), and monosodium glutamate (E 621) (0.05%). Spices and additives for sample 3 were: spice mixture with celery (1.4%), NaCl (0.75%), spices (0.5%), a flavor enhancer (0.15%), Acerola cherry concentrate (0.1%).

Texture profile analysis (TPA) was performed as described by Bourne (BOURNE [19]) at room temperature, using TA. XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable MicroSystems, Godalming, UK) equipped with a standard cylindrical

plate of 75 mm in diameter. Samples, which were 2 cm thick, were compressed twice to 50% of their original thickness at a constant test speed of 1 mm/s. The following parameters were determined: hardness, springiness, cohesiveness and chewiness. 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. Chewiness was obtained by multiplying hardness, cohesiveness and springiness. A Warner-Bratzler (WB) shear test (DE HUIDOBRO & al. [20]) was carried out using a WB shear V blade. The crosshead speed was 1.5 mm/s. The following shear parameters were calculated from the force–deformation curves: maximum shear peak force (highest peak detected during the test – firmness), and total area (fitting area plus shear area) as the total amount of work required to cut through the sample.

Color of sausages was determined on samples of a minimum thickness of 2.5 cm, and it was measured in triplicate on the surface of each sample. The CIE  $L^*$  (lightness), CIE  $a^*$  (redness) and CIE  $b^*$  (yellowness) color coordinates (CIE, 1976) were determined using MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan) using D-65 lighting, a  $2^\circ$  standard observer angle and an 8-mm aperture in the measuring head. The Chroma Meter was calibrated using a Minolta calibration plate (No. 11333090;  $Y=92.9$ ,  $x=0.3159$ ;  $y=0.3322$ ).

Sample for sensory evaluation were previously prepared for consumption (hot samples). Six trained panellists sensory evaluated samples, by five attributes (appearance, cross section, color on the cutting, odor and taste, and texture) in order to determine differences between examined sausages. An evaluation was performed according to quantitative descriptive analysis, using a scale from 0 (atypical product) to 5 (extraordinary, typical, optimal quality), with a sensitivity threshold of 0.5 points (STONE & SIDEL [21], ISO 4121 [22]). The overall sensory quality of sausages was evaluated according to the following expression: Overall sensory quality = (appearance of sausage x 3 + cross section x 3 + color on the cutting x 4 + odor and taste x 6 + texture x 4) / 20.

Descriptive statistical analyses of all the obtained results were expressed as the mean  $\pm$  standard deviation (SD). All sausage samples were analysed in triplicate. Principal component analysis (PCA) was used to discover the possible correlations among measured parameters, while Cluster analysis (CA) was used to classify objects into groups. PCA analyses of the obtained results were performed using Stat Soft Statistica 10.0<sup>®</sup> software.

Standard scores is one of the most widely used technique to compare various characteristics of various samples determined using multiple measurements, where samples are ranked based on the ratio of raw data and the extreme values of the measurement used. Since the scale (and sometimes the units) of the data acquired from various samples, using measuring methods are different, the data in each data set should be transformed into normalised scores, (dimensionless quantities), according to following equations:

$$\bar{x}_i = 1 - \frac{\max_i x_i - x_i}{\max_i x_i - \min_i x_i}, \quad \forall i \text{ in case of "the higher, the better" criteria, or}$$

$$\bar{x}_i = \frac{\max_i x_i - x_i}{\max_i x_i - \min_i x_i}, \quad \forall i, \text{ in case of "the lower, the better" criteria.}$$

where  $x_i$  represents the raw data. The normalized scores of a sample of different measurements, when averaged, give a single unit less value, termed as SS, which is a specific combination of data from different measuring methods with no unit limitation. This approach also enables the ease of employing some other sets of data to this elaboration.

### 3. Results and Conclusions

The changes in firmness and work of share in different formulations of sausages are shown in the Table 1.

**Table 1.** Texture properties (Warner-Bratzler shear test) of cold (C) and re-heated (T) control and test sausage samples

Sample	Firmness (N)	Work of share (N*s)	SS
1C	1.14±0.24 <sup>ab</sup>	12.96±1.73 <sup>bc</sup>	0.08
2C	1.81±0.09 <sup>c</sup>	22.15±0.46 <sup>c</sup>	0.92
3C	1.39±0.09 <sup>a</sup>	14.78±0.94 <sup>abcd</sup>	0.32
4C	1.43±0.21 <sup>a</sup>	17.05±1.39 <sup>a</sup>	0.45
5C	1.39±0.13 <sup>a</sup>	17.00±0.98 <sup>a</sup>	0.42
1T	1.05±0.08 <sup>b</sup>	12.50±1.14 <sup>b</sup>	0.00
2T	1.79±0.17 <sup>c</sup>	24.01±1.15 <sup>c</sup>	0.98
3T	1.26±0.28 <sup>ab</sup>	15.31±2.42 <sup>acd</sup>	0.26
4T	1.23±0.10 <sup>ab</sup>	14.19±1.15 <sup>bcd</sup>	0.19
5T	1.17±0.14 <sup>ab</sup>	16.13±1.58 <sup>ad</sup>	0.24
<b>Polarity</b>	+	+	
<b>CV(%)</b>	14.7	15.8	

<sup>a-d</sup> values with the different letter within a column are statistically different at the  $p < 0.05$  level (according to post-hoc Tukey's HSD test)

The minimum values of firmness were determined for the control group, regarding both cold and re-heated sausages (1.14 N, 1.05 N, respectively), while the highest firmness was observed for the sausages with the addition of  $\omega$ -3 fatty acids (1.81 for 2C; 1.79 N for 2T group). The highest work of share, also was observed for sausages with  $\omega$ -3 fatty acids addition (22.15 N\*s and 24.01 N\*s, for 2C and 2T group, respectively). The minimum values of work of share were also observed for control samples (12.96 N\*s for cold and 12.50 N\*s for re-heated samples). Coefficients of variation showed relatively high values reaching 14.7 and 15.8%, for firmness and work of share, respectively. The maximum of standard score regarding firmness and the work of share was observed for sample 2, re-heated sample.

Table 2 shows the changes in hardness, springiness, cohesiveness, gumminess, and chewiness in different formulations of sausages. The initial hardness of cooked sausage was 5945.47 g (1C), while the hardness for re-heated sausages dropped to 5246.18 g (1T). Apart of these values, the highest value of hardness was observed with the addition of phytosterol (5401.65 g for 5C, and 4770.24 g for 5T sausage). There were no statistically significant ( $P > 0.05$ ) differences in springiness and cohesiveness for observed samples. Springiness values were in the range from 0.73 to 0.81, while the measured cohesiveness values were between 0.76 and 0.79. The maximum values of gumminess were observed for control samples (4518.37g for 1C and 3984.25g for 1T sausages). Also, the samples containing phytosterol had high values for gumminess, 4145.86 for sample 5C and 3742.24 for sample 5T. The maximum of chewiness was observed for control sausages (3628.95g for 1C and 3118.24g for 1T), but the samples with phytosterol or  $\omega$ -3 fatty acids addition also showed high values of chewiness. Coefficients of variation showed relatively high values reaching 14.7, 12.3, 6.6, 14.9, and 14.1%, for hardness, springiness, cohesiveness, gumminess and chewiness, respectively. The maximum of standard score regarding texture properties (TPA test) was observed for control cold sample (1C).

**Table 2.** Texture properties (TPA test) of cold (C) and re-heated (T) control and test sausage samples

Sample	Hardness (g)	Springiness	Cohesiveness	Gumminess (g)	Chewiness (g)	SS
1C	5945±420 <sup>b</sup>	0.80±0.04 <sup>a</sup>	0.76±0.02 <sup>a</sup>	4518±226 <sup>b</sup>	3628±376 <sup>b</sup>	0.78
2C	5306±187 <sup>ab</sup>	0.81±0.03 <sup>a</sup>	0.77±0.01 <sup>a</sup>	4065±148 <sup>ab</sup>	3282±141 <sup>ab</sup>	0.72
3C	3984±277 <sup>c</sup>	0.81±0.02 <sup>a</sup>	0.78±0.01 <sup>a</sup>	3118±200 <sup>c</sup>	2526±225 <sup>ac</sup>	0.60
4C	4863±911 <sup>abc</sup>	0.74±0.08 <sup>a</sup>	0.78±0.03 <sup>a</sup>	3756±551 <sup>abc</sup>	2826±747 <sup>ab</sup>	0.53
5C	5401±599 <sup>ab</sup>	0.78±0.04 <sup>a</sup>	0.77±0.01 <sup>a</sup>	4145±392 <sup>ab</sup>	3253±476 <sup>ab</sup>	0.68
1T	5246±961 <sup>ab</sup>	0.77±0.07 <sup>a</sup>	0.76±0.05 <sup>a</sup>	3984±684 <sup>ab</sup>	3118±726 <sup>ab</sup>	0.59
2T	4686±200 <sup>ac</sup>	0.75±0.05 <sup>a</sup>	0.78±0.01 <sup>a</sup>	3637±134 <sup>ac</sup>	2736±244 <sup>ab</sup>	0.52
3T	3909±847 <sup>c</sup>	0.74±0.06 <sup>a</sup>	0.79±0.02 <sup>a</sup>	3090±617 <sup>c</sup>	2322±652 <sup>ac</sup>	0.47
4T	2668±189 <sup>d</sup>	0.74±0.05 <sup>a</sup>	0.79±0.01 <sup>a</sup>	2101±126 <sup>d</sup>	1566±189 <sup>c</sup>	0.21
5T	4770±891 <sup>abc</sup>	0.73±0.09 <sup>a</sup>	0.79±0.05 <sup>a</sup>	3742±545 <sup>abc</sup>	2758±744 <sup>ab</sup>	0.56
<b>Polarity</b>	+	+	+	+	+	
<b>CV(%)</b>	14.7	12.3	6.6	14.9	14.1	

<sup>a-d</sup> values with the different letter within a column are statistically different at the  $p < 0.05$  level (according to post-hoc Tukey's HSD test)

Table 3 shows the changes in color characteristics in different formulations of sausages, expressed in CIE  $L^*a^*b^*$  color coordinates. The initial redness color of cold sausage was 17.30 (1C), while for the re-heated cooked sausage redness increased to 17.69 (1T).

**Table 3.** Color characteristics of cold (C) and re-heated (T) control and test sausage samples

Sample	$a^*$	$b^*$	$L^*$	SS
1C	17.30±0.27 <sup>c</sup>	13.34±0.51 <sup>ab</sup>	67.92±1.02 <sup>bc</sup>	0.47
2C	15.66±0.05 <sup>d</sup>	13.46±0.11 <sup>ab</sup>	68.05±0.04 <sup>bc</sup>	0.36
3C	13.76±0.12 <sup>a</sup>	13.49±0.09 <sup>ab</sup>	71.21±0.15 <sup>a</sup>	0.44
4C	14.38±0.19 <sup>bc</sup>	14.21±0.19 <sup>bc</sup>	70.62±0.94 <sup>a</sup>	0.60
5C	14.60±0.03 <sup>c</sup>	13.93±0.17 <sup>abc</sup>	70.59±0.60 <sup>a</sup>	0.55
1T	17.69±0.25 <sup>c</sup>	13.73±0.33 <sup>abc</sup>	66.65±0.55 <sup>b</sup>	0.48
2T	15.62±0.09 <sup>d</sup>	13.30±0.12 <sup>ab</sup>	66.67±0.33 <sup>b</sup>	0.23
3T	14.03±0.21 <sup>ab</sup>	12.98±0.09 <sup>a</sup>	70.29±0.46 <sup>a</sup>	0.29
4T	13.70±0.19 <sup>a</sup>	14.64±0.93 <sup>c</sup>	68.48±0.62 <sup>c</sup>	0.47
5T	14.33±0.08 <sup>bc</sup>	13.89±0.19 <sup>abc</sup>	69.65±0.35 <sup>ac</sup>	0.46
<b>Polarity</b>	+	+	+	
<b>CV(%)</b>	1.6	6.3	1.5	

<sup>a-d</sup> values with the different letter within a column are statistically different at the  $p < 0.05$  level (according to post-hoc Tukey's HSD test)

Apart of these values, the highest value of redness was observed for samples with the  $\omega$ -3 fatty acids addition (15.66 and 15.62 for 2C and 2T, respectively). The maximum of yellowness color was observed for samples with tocopherol addition (14.21 for 4C and 14.64 for 4T). Samples with natural additives had the highest lightness value ( $L^*$ ) (71.21 3C and 70.29 for 3T). Coefficients of variation showed very low values reaching 1.6, 6.3 and 1.5%,

for  $a^*$ ,  $b^*$  and  $L^*$  value, respectively. The maximum of standard score regarding color properties was observed for the sample with tocopherol addition (sample 4C).

The effects of different additives addition to the sensory characteristics (appearance, cross section, color, odor and taste, and texture) of re-heated cooked sausages are shown in Table 4. Significantly ( $P < 0.05$ ) the highest scores for appearance had control (4.58) and samples with phytosterol addition (4.33).

**Table 4.** Sensory characteristics of re-heated control and test sausage samples

Sample	Appearance	Cross section	Color on the cutting	Odor and taste	Texture	Overall sensory quality
1T	4.58±0.14 <sup>b</sup>	4.08±0.14 <sup>cd</sup>	4.17±0.14 <sup>b</sup>	3.17±0.29 <sup>a</sup>	3.75±0.00 <sup>b</sup>	3.83
2T	3.92±0.14 <sup>a</sup>	4.25±0.25 <sup>d</sup>	4.33±0.14 <sup>b</sup>	3.58±0.14 <sup>a</sup>	4.00±0.00 <sup>bc</sup>	3.97
3T	3.75±0.00 <sup>a</sup>	3.58±0.29 <sup>bc</sup>	3.67±0.14 <sup>a</sup>	3.50±0.25 <sup>a</sup>	3.92±0.14 <sup>bc</sup>	3.67
4T	4.00±0.00 <sup>a</sup>	3.00±0.00 <sup>a</sup>	3.67±0.14 <sup>a</sup>	3.25±0.25 <sup>a</sup>	3.17±0.14 <sup>a</sup>	3.30
5T	4.33±0.14 <sup>b</sup>	3.42±0.14 <sup>ab</sup>	3.75±0.00 <sup>a</sup>	3.42±0.14 <sup>a</sup>	4.12±0.13 <sup>c</sup>	3.76
<b>Optimal</b>	5	5	5	5	5	
<b>CV(%)</b>	3.69	8.06	3.94	9.12	4.56	

<sup>a-d</sup> values with the different letter within a column are statistically different at the  $p < 0.05$  level (according to post-hoc Tukey's HSD test)

Control sample and sample with  $\omega$ -3 fatty acids addition had significantly or numerically the highest scores for cross section and color on the cutting. There was no statistically significant difference ( $P > 0.05$ ) in odor and taste between cooked sausages with different additives addition. Scores for odor and taste ranged from 3.17 (control) to 3.58 (sample 2). Samples with phytosterol addition had the highest and the samples with tocopherol addition the lowest score for texture (4.12 and 3.17, respectively).

Coefficients of variation ranged from 3.69% (for appearance) to 9.12% (for odor and taste). Overall sensory quality was the highest for samples with  $\omega$ -3 fatty acids addition (3.91) and the lowest for samples with tocopherol addition (3.30).

A dendrogram of cooked sausages samples, using a complete linkage as an amalgamation rule and the city block (Manhattan) distance as a measure of the proximity between samples is shown in Figure 1. The dendrogram based on texture and color characteristics of experimental data showed the proper distinction between cold and re-heated sausages of different samples. The variability among samples is due to chemical composition. As shown in Figure 1 there was similarity in the samples T1, C1, C2 and C5 on the dendrogram, while the other branch was encampments the samples: T2, T3, T5, C3 and C4.

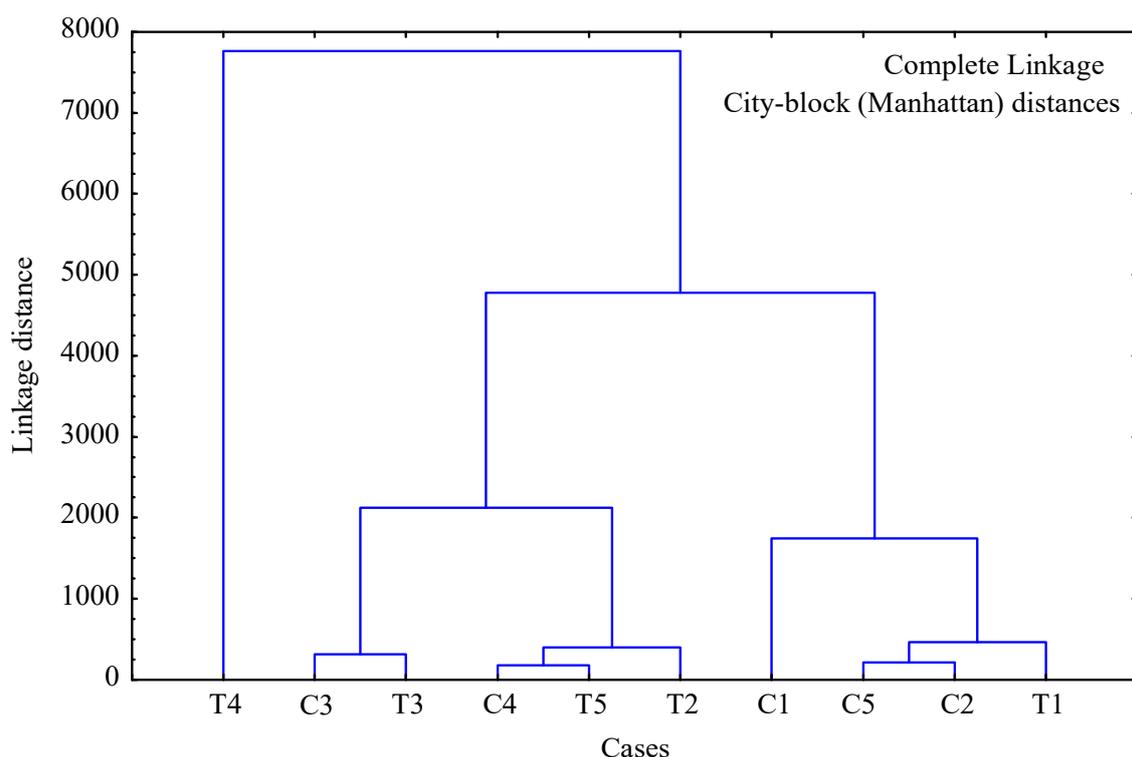


Figure 1. Complete-linkage dendrogram for cold (C) and re-heated (T) control and test sausage samples

The PCA allows a considerable reduction in a number of variables and the detection of structure in the relationship between measuring parameters and different varieties of processing parameters that give complimentary information. The full auto-scaled data matrix consisting of measurement results for cold (C) and re-heated (T) samples with the addition of different additives were submitted to PCA. For visualizing the data trends and the discriminating efficiency of the used descriptors, a scatter plot of samples using the first two principal components (PCs) issued from PCA of the data matrix is obtained (Fig. 2). As can be seen, there is a neat separation of the 5 samples (cold and re-heated) of sausages with differentiation of additives.

The PCA of the presented data explained that the first three components accounted for 83.03% of the total variance (50.03, 20.41 and 12.59%, for the first, the second and the third principal component, respectively) in the ten variables ( $L^*$ ,  $a^*$ ,  $b^*$ , hardness, springiness, cohesiveness, gumminess, chewiness, firmness and toughness). Considering the map of the PCA performed on the data, cohesiveness (which contributed 16.39% of total variance, based on correlations) exhibited positive scores according to first principal component, whereas hardness (17.53%), gumminess (17.06%), chewiness (18.16%), and  $a^*$  value (14.39%) showed a negative score values according to first principal component (Figure 2). The positive contribution to the second principal component calculation was observed for: work of shear (44.96% of total variance, based on correlations) and firmness (45.67%). The positive contribution to the third principal component was obtained for  $L^*$  value (60.47% of total variance, based on correlations), while the negative influence was observed for  $a^*$  value (13.78%).

The increased springiness, hardness, chewiness and gumminess could be expected for samples with  $\omega$ -3 fatty acid addition, while cohesiveness is more likely to be found in samples with tocopherol and phytosterol addition. Samples with  $\omega$ -3 fatty acid addition showed an increased toughness and firmness.

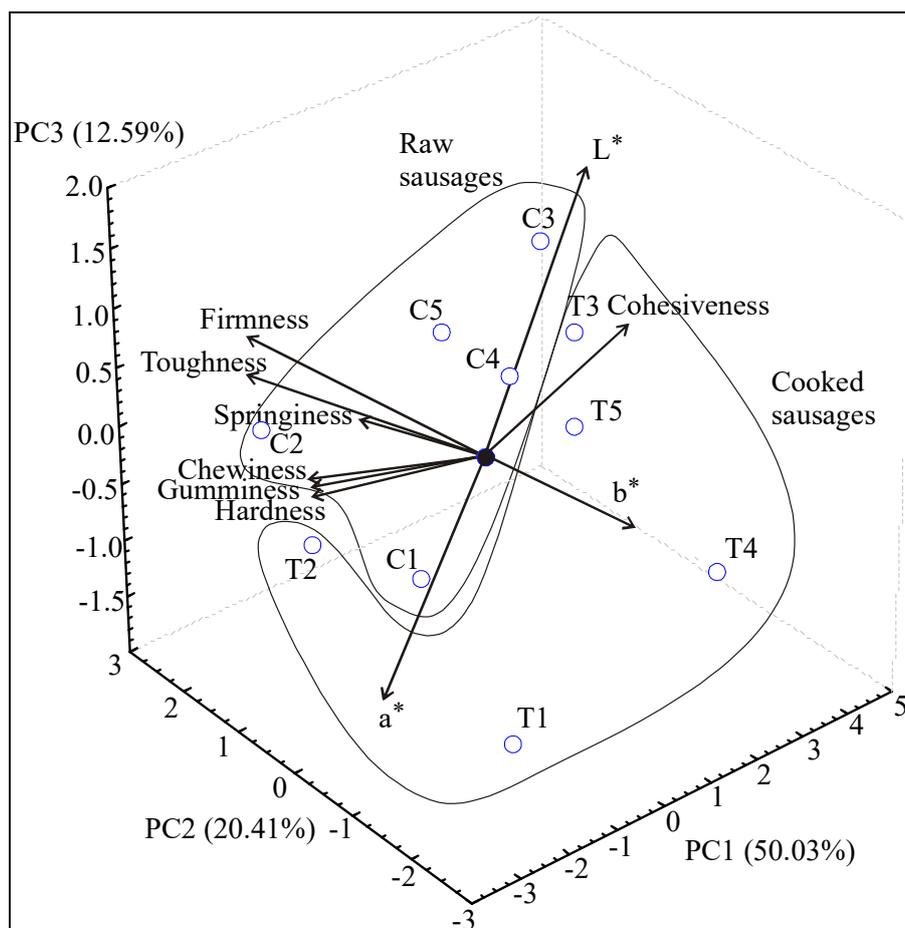


Figure 2. Biplot of texture properties, color and sensory characteristics of cold (C) and re-heated (T) control and test sausage samples

#### 4. Conclusion

Samples with  $\omega$ -3 fatty acid addition showed an increased firmness and the maximum of standard score regarding texture properties determined by Warner-Bratzler shear test, while the maximum of standard score regarding texture properties determined by TPA test was observed for control cold sample (1C). The maximum of standard score regarding color properties was observed for the sample with tocopherol addition. Samples of re-heated cooked sausages with the addition of  $\omega$ -3 fatty acid were evaluated with the highest overall sensory quality, while other samples had uniform overall sensory quality.

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