Bulgarian Journal of Agricultural Science, 23 (No 2) 2017, 338–347
Agricultural Academy

IMPROVEMENT OF TEXTURE PROFILE ATTRIBUTES OF COOKED SAUSAGE TYPE "KRENVIRSH"

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Abstract

Chorbadzhiev, P., G. Zsivanovits, D. Gradinarska, K. Danov and K. Valkova-Jorgova, 2017. Improvement of texture profile attributes of cooked sausage type "krenvirsh". *Bulg. J. Agric. Sci.*, 23 (2): 338–347

Aim of this work is determining the textural and microbiological properties of cooked sausage samples type "krenvirsh" and studying the influence of different collagen preparations addition on their quality attributes. The studies were conducted with experimental samples, during the production process of which two collagen preparations were added: CPS-C – commercial one, and CPS-U – laboratory produced from pork skins by mechanical treatment, both in concentrations of 15.00, 25.00 and 35.00 $g.kg^{-1}$, compared to raw meat. Control samples, without addition of collagen preparations, were also produced. Experimental data shows growing preparations influence on investigated sausage characteristics by increasing quantity of their addition. Lower concentrations lead to improved texture characteristics, as such advantage is demonstrated when CPS-U was used, due to its moderate influence on sausages. Addition of CPS-U in quantity of 15.00 $g.kg^{-1}$ has a beneficial effect, resulting in formation of typical for the high quality products denser structure by slight increase of hardness and springiness, together with releasing of chewiness values, similar to those of the control samples. Quantities of 25.00 and 35.00 $g.kg^{-1}$ induce significant changes in product texture profile, which is clearly expressed in values obtained for hardness and chewiness. Microbiological analysis of control and experimental samples indicates slight differences between themselves.

Key words: cooked sausages, various collagen preparations, texture modification

Introduction

Meat products texture constitutes an important part of their organoleptic characteristics. As one of the main factors related to their quality attributes, it has a direct impact on consumers, because its image is formed directly through the senses (Hathwar et al., 2012). The texture is „response" to tactile perceptions of a person to received external stimulation caused by contact between a part of the body and a food (Bourne, 2002).

Collagen proteins express proven gel-forming and viscous properties that turn them into raw material widely used in meat industry, with the purpose to improve technological properties as water retention, stabilization of meat „emulsions", gelling, etc. (Smith, 1988; Schilling et al., 2003; Prabhu et al., 2006). Essential role for the wide variety of functional properties – gelling and emulsifying ability, surface activity, film-forming ability, etc., plays the typical collagen molecules structure called „triple helix". The influence of these proteins on products texture characteristics is object of many researches.

It has been found that the addition of low quantities collagen preparation has a positive effect due to its gelling

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and water-binding abilities, resulting in reduction of thermal shrinkage and improving components of cooked sausage texture profile, together with keeping sensory characteristics unaltered (Prabhu and Doerscher, 2000; Schilling et al., 2003; Doerscher et al., 2004; Prabhu et al., 2006; Indzhelieva, 2015). Similar to these results were obtained by Choe et al. (2013); Sarbon et al. (2013); Prestes et al. (2013).

Bonifer et al. (1996) observed variations in texture profile of cooked sausage type "bologna" due to use of high quantity collagen preparation. Similar findings were reported by Buyck et al. (1982); Swatland and Barbut (2007), indicating the high fat content of collagen preparations as the main reason for the negative impact. According to other researchers, collagen preparations exhibit slight, often not statistically significant, influence on meat products sensory profile (Pereira et al., 2011).

The aim of this study is to conduct a comparative analysis on influence of two types collagen preparations, incorporated in varying amounts, on textural, microbiological and some physicochemical characteristics of structureless cooked sausage type „krenvirsh".

Materials and Methods

Preparation of cooked sausages

The studies were conducted with structureless cooked sausage type "krenvirsh", using the following raw meats – chilled pork ham – protein content 188.00 $g.kg^{-1}$, fat content 51.00 $g.kg^{-1}$, water content 754.00 $g.kg^{-1}$, pH6.15; and chilled pork ribs – protein content 120.00 $g.kg^{-1}$, fat content 392.00 $g.kg^{-1}$, water content 483.00 $g.kg^{-1}$, pH6.13. Sausages were produced using the following recipe: pork lean meat – 700.00 $g.kg^{-1}$, pork fat meat – 300.00 $g.kg^{-1}$. Compared to raw meats also were added: nitrite salt – 18.00 $g.kg^{-1}$, sodium polyphosphate – 3.00 $g.kg^{-1}$, ascorbic acid – 0.20 $g.kg^{-1}$, white pepper – 3.00 $g.kg^{-1}$, red pepper – 1.00 $g.kg^{-1}$, nutmeg – 0.50 $g.kg^{-1}$, ginger – 0.20 $g.kg^{-1}$, caraway – 0.20 $g.kg^{-1}$. Quantities of flake ice and collagen preparation, as well as the flow diagram of sausage production process, are presented in Figure 1.

Sausages are produced in Department of "Meat and fish technology", University of Food Technologies, Plovdiv. Prior to meat batter preparation all subcutaneous and intramuscular fat and visible connective tissues were removed. Raw meat was cut into pieces, placed in a cutter machine and treated with salting materials to produce a fine, homogenous mass. Flake ice and collagen preparations were added during processing. The meat batter was stuffed into artificial polyamide casings and subjected to heat treatment as follows: 1) 10 min at 55°C, 2) 15 min at 65°C, 3) 15 min at 75°C, 4) at 80°C to 72°C core temperature.

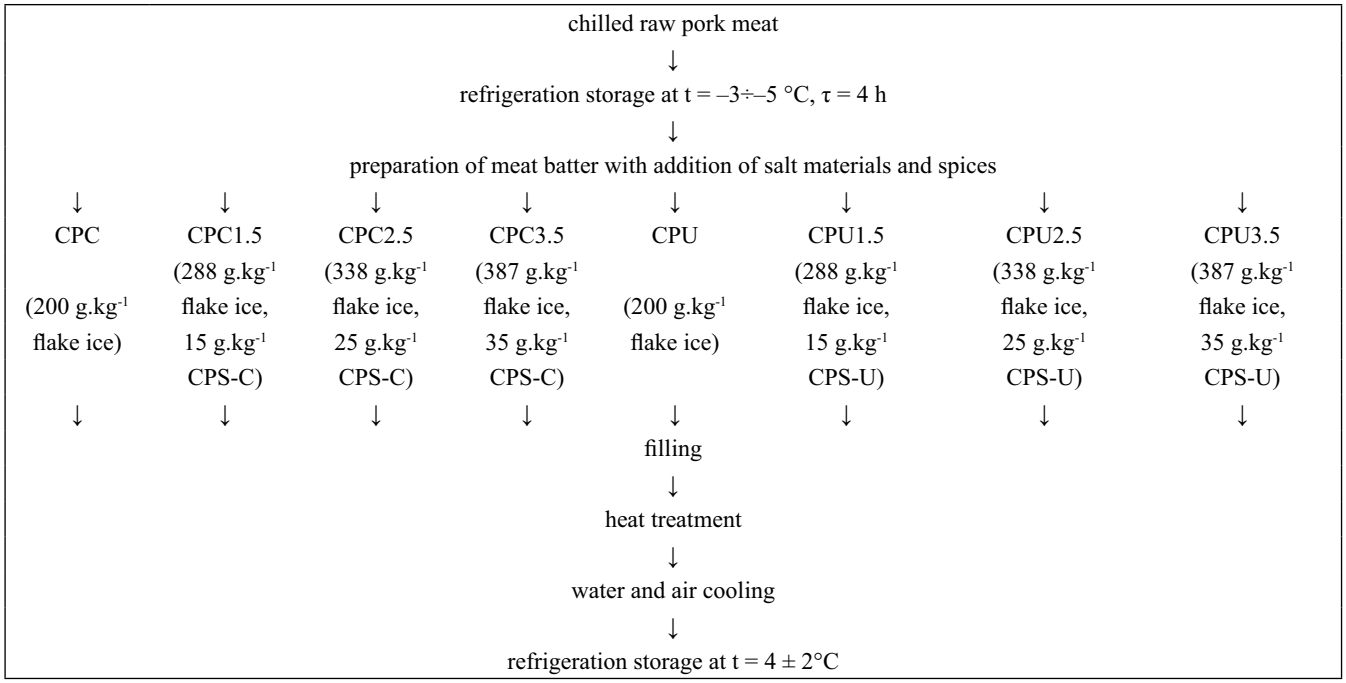

Fig. 1. Production process of cooked sausage type "krenvirsh"

Physicochemical analyses

The pH values of cooked samples were determined using pH meter MS 2004 (Microsyst, Bulgaria); sample aqueous extracts in proportion sample/water 1:9 (w/v) were previously prepared. During the extraction period, which lasts 20 min, the mixtures were periodically stirred, and then the extracts were filtered through filter paper prior to the measurement. Water activity of tested samples was measured using instrument LabSwift-a_w system (Novasina AG, Switzerland) at 25°C.

Texture profile analysis (TPA)

Prior to analysis sausages were left for 60 min at room temperature. The analysis was conducted according to the method described by Bourne (1978) with some modifications. Therefor after peeling of sausages the same were cut with a template to form cube with 20 mm side. The samples were compressed twice to 50% of their original height using a texture analyzer TA-XT.Plus (Stable Micro Systems, Surrey, Great Britain), equipped with 25 kg load cell and a flat cylindrical aluminum probe (50 mm diameter). Relaxation time between two compressions was set to 3 sec. Analyzes were performed with following parameters: speed of the working body before reaching the sample – 2 $mm.s^{-1}$; test speed – 2 $mm.s^{-1}$; withdrawal speed – 2 $mm.s^{-1}$.

Curves for force against time were obtained. Texture profile analysis components are presented as follows: 1. hardness (N), maximum force registered during the first compression; 2. springiness, ratio of the distance of detected height during the second compression divided by the original compression distance; 3. adhesiveness (N.mm), the negative force registered during the first compression, representing the work necessary to pull the compressing plunger away from the sample; 4. cohesiveness, the ratio of positive work registered during the second compression to that registered during the first compression; 5. chewiness, the product of hardness, cohesiveness and springiness.

Microbiological analysis

Sample preparation was conducted in accordance with EN ISO 6887-2:2004. Following microbiological parameters were examined:

Product hygiene and shelf life assessing:

- Total plate count (aerobic mesophilic microorganisms) (TPC) – according to EN ISO 4833-2: 2013
- Determination of coliforms count – by preparing inoculations of 1 cm^3 of decimal dilutions on HiCrome Coliform Agar w/SLS (HiMedia), incubation for 48 h, t = 37°C – validated method of ISO 4832:2002
- Determination of yeasts and molds count – ISO 7954:2002
- Determination of sanitary indicative enterococci – by preparing inoculations of 1 cm^3 of previously prepared decimal dilutions on Slanetz and Bartley Medium agar (HiMedia), incubation for 48 h, t = 37°C

Product safety assessing:

- Analysis for presence of *Salmonella spp.* – According to EN 12824:2002
- Analysis for presence of *Listeria monocytogenes* – validated method, ISO 11290-1 (Scotter et al., 2001).

The results, indicating hygiene and shelf life, are expressed as $\log_{10}$cfu (colony forming units) per g of sausage and these defining safety – by the presence (resp. the absence) of pathogenic bacteria in 25 g of sausage.

Statistical analysis

Analysis of variance (one-way ANOVA) with significance level of $P \leq 0.05$ was conducted (Draper et al., 2014). Differences between values lower than 0.05 were considered statistically significant. Tukey's test for multiple comparisons between samples was used. Statistical procedures were performed using Microsoft Excel 5.0 and SPSS 16 software.

Results and Discussion

Physicochemical parameters

Experimental data obtained for pH-value and water activity of the samples is presented in Table 1.

Table 1
pH- and water activity-values of structureless cooked sausage type "krenvirsh"

Sample	pH-value, - (24^{th}hour)	pH-value, - (4^{th} day)	water activity, (4^{th} day)-
CPC	$6.54^{c,d,y}$	$6.60^{b,c,z}$	0.979^{a}
CPC1.5	$6.56^{c,z}$	$6.61^{b,z}$	0.973^{b}
CPC2.5	$6.64^{b,y}$	$6.69^{a,z}$	0.967^{d}
CPC3.5	$6.71^{a,z}$	$6.74^{a,z}$	0.966^{e}
CPU	$6.47^{e,f,z}$	$6.50^{d,z}$	0.980^{a}
CPU1.5	$6.49^{f,y}$	$6.59^{b,c,z}$	0.970^{c}
CPU2.5	$6.50^{d,e,f,z}$	$6.54^{c,d,z}$	0.966^{d}
CPU3.5	$6.48^{f,y}$	$6.61^{b,z}$	0.962^{f}

a-f – means with same superscripts are not significantly different ($P > 0.05$)
z-y – means of same probe, obtained on 24^{th} hour and 4^{th} day, with same superscripts are not significantly different ($P > 0.05$)

Trend of parameter value increase together with increase of added collagen preparation and water amounts during 1st day of storage was established. The use of commercial preparation leads to much more pronounced increase of pH-values. Sample CPC3.5 exhibits highest value ($P < 0.05$), followed by that of sample CPC2.5 ($P < 0.05$). The dynamic increase of parameter values, which was found as typical for samples produced with addition of CPS-C, was not observed when CPS-U was added. CPS-U-addition is associated with values in the range of 6.47 ÷ 6.50. These close values, although some of them are statistically significant from each other, reaffirm the view, according to which hydrogen ion concentration of samples, produced with CPS-C, increases due to its higher starting pH-value (6.00 ÷ 8.00).

Data obtained on the 1st day of storage shows that samples reveal pH-values, which are normal for this type of products – 5.80 ÷ 6.50 (Sielaff, 1996; Baumgartner, 2001), although they are close to upper limit. Established approaching to these high values is due to the aforementioned reasons. These high parameter values require appropriate refrigerated storage (0÷4°C), since sausages afford favorable factors for growth of microorganisms – pH6.0 ÷ 8.0 and high water content (Adams and Moss, 2008). Properly conducted heat treatment and reaching internal temperature of 72°C (Feiner, 2006), as well as proper microbiological control of spices and additives (Jay et al., 2008), prevent sausages from unwanted microbiological changes during their four-day storage.

Table 1 presents values of samples hydrogen ion concentration, determined on the 4th day of the storage. Highest values reveal samples CPC2.5 and CPC3.5 ($P < 0.05$), they are not significantly different from one another ($P > 0.05$). It should be noted that the increase of control samples values is much more moderately. The higher pH-values, observed on the fourth day, can be explained with proteolytic changes in protein fraction, accompanying the storage period. Destruction of the tertiary and quaternary protein molecules structure is linked to the hydrogen bonds destruction, which plays an important role in their maintenance (Feiner, 2006). Destroyed bonds lead to an increase in number of positively charged hydrogen ions, and this has a direct impact on pH-values. Greater difference between pH-values, measured on 1st and 4th day, of samples with higher protein content was determined, due to the addition of collagen preparation during production process. The thermal treatment of sausages is associated with denaturation changes in collagen molecules at temperatures above 56-60°C. These lead also to disruption of hydrogen bonds and thus separation of positively charged hydrogen ions in the medium. This may explain the great difference in pH-value of the sausages in the beginning and in the end of storage period.

The comparative analysis of samples values, obtained on the 1st and the 4th day, showed that storage period has moderate influence on pH. Samples CPC1.5, CPC3.5, CPU, CPU2.5 showed same values on 1st and 4th day ($P > 0.05$). Other samples differ statistically ($P < 0.05$).

Experimental data, obtained from the conducted analysis for determination the influence of CPS-C and CPS-U on water activity of produced samples, shows that control samples, CPC and CPU, reveal highest values, respectively 0.979 and 0.980 ($P > 0.05$). According to Feiner (2006) these values are typical for the group of cooked sausages, whose water activity ranges from 0.970 to 0.980. Inverse relationship between the value of examined parameter and amount of the added collagen preparation is observed in experimental samples. This dependence, as well as the significant differences in regard to parameter values of control and experimental samples, are evidence of protein preparations influence. Collagen proteins have the ability by contact with water to bind it chemically through swelling of their protein matrix (Osburn and Mandigo, 1998; Schilling et al., 2003; Prabhu et al., 2006; Tarté, 2009). This strong binding of water undoubtedly leads to reduction of its percentage in product composition, which in turn is expressed in reduction of water activity value.

Lowest water activity values are obtained in samples CPC3.5 and CPU3.5, respectively 0.966 and 0.960; the difference between them is statistically significant. Very close values are established when quantity of the added preparation is 25.00 $g.kg^{-1}$ ($P > 0.05$). Experimental samples, produced with addition of CPS-U, released lower water activity-values, compared to samples produced with same amount of CPS-C. As opposed to aforementioned results in other studies conducted with cooked sausages produced with collagen fibers addition, it has not been established statistically significant difference in relation to water activity values of control and experimental samples (Pereira et al., 2011). This confirms the importance of preparation type with regard to a_w-changes and releasing of values, favorable for product microbial safety.

Texture profile analysis (TPA)

Table 2 presents instrumentally obtained data for products texture profile components.

In terms of texture profile analysis, hardness can be described as the maximum force applied during first compression of sample, respectively its chewing. Experimentally obtained data indicates significant differences between samples. Most pronounced hardness – 81.78 N, was established in sample CPC3.5 ($P < 0.05$). The increase of

Table 2
Influence of collagen preparations on texture profile components of the final product

Sample	Hardness, N	Springiness, N-	Adhesiveness, N.mm	Cohesiveness, N.mm-	Chewiness, N
CPC	40.93^{g}	0.76^{d}	-0.59^{a,b}	0.53^{c}	16.40^{f}
CPC1.5	56.50^{e}	0.86^{b}	-0.63^{a}	0.60^{b}	29.28^{d}
CPC2.5	70.25^{c,d}	0.87^{b}	-0.38^{c}	0.67^{a}	41.11^{b}
CPC3.5	81.78^{a}	0.92^{a}	-0.37^{c}	0.67^{a}	50.02^{a}
CPU	42.13^{g}	0.76^{d}	-0.47^{b,c}	0.53^{c}	16.86^{f}
CPU1.5	50.30^{f}	0.81^{c}	-0.37^{c}	0.55^{c}	22.37^{e}
CPU2.5	66.61^{d}	0.86^{b}	-0.34^{c}	0.62^{a,b}	35.83^{c}
CPU3.5	73.43^{b,c}	0.89^{a,b}	-0.45^{b,c}	0.66^{a}	43.06^{b}

a-g – means with same superscripts within a column are not significantly different ($P > 0.05$)

parameter value can be explained with the possibility of formation of large and unevenly distributed collagen aggregates in thermally denatured protein network, grown up as a result of high amount added collagen preparation. High parameter value is obtained also in sample CPU3.5 – 73.43 N, this fact gives rise to do an assumption about existence of possible proportional relationship between parameter value and amount of added preparation. Such relationship is observed but it should be noted that CPS-U-addition, compared to the addition of same amount CPS-C, leads to formation of lower parameter value. Except the addition of high amounts collagen preparation, which leads to increase of sausage protein content, reduced fat and increased water content, as well as the possibility of formation of high number protein-protein interactions, can be indicated as main factors influencing hardness increase (Youssef and Barbut, 2010; Pereira et al., 2011; Choe et al., 2013). Samples produced with lowest level of collagen preparation addition, CPC1.5 and CPU1.5, exhibit clearly expressed differences in terms of their hardness values, as the latter ones are very similar to control samples values. However, CPU1.5 releases value that is significantly different from those of CPC and CPU ($P < 0.05$), and the observed slight increase of hardness probably would have a positive impact on sausage organoleptic characteristics. Such an improvement of cooked sausages texture profile, result of the addition of low concentrations collagen preparation, was reported by Freitas et al. (2004).

Other important texture profile component is springiness. Expressed as ratio, springiness values may vary from 0 to 1. The aim of springiness determination is establishing the extent to which the measured sample can restore its original height after its deformation during the first compression, respectively first bite. Besides product structure, other important recovery factor is the period of relaxation between two cycles, respectively sample mastication.

Samples CPC3.5 and CPU3.5 release again highest established parameter values, respectively 0.92 and 0.89. These values do not differ significantly ($P > 0.05$), same trend was established for samples, produced with amount of 25.00 g.kg^{-1} collagen preparation addition, CPC2.5 and CPU2.5, which release next high values, respectively 0.87 and 0.86 ($P > 0.05$). Taking into account data obtained from the conducted analyses, it should be noted that a trend was established, according to which CPS-C and CPS-U reveal similar level of influence on product characteristics. In case of their use in higher amounts (≥25.00 g.kg^{-1}), quantity can be described as factor having greater influence, compared to preparation type. Unlike the reported by Pereira et al. (2011), decrease of springiness values affected by the increasing amount of added water, produced samples do not follow this trend, according to experimentally obtained data in this study. The established increase of springiness values of control samples represents direct consequence of product formulations, according to which the increase of added water quantity is followed by such of final product protein content.

The values revealed for CPC1.5 and CPU1.5 ($P < 0.05$) are in contrast to the observed trend. In case of low amounts collagen preparation usage crucial for its influence on produced sausages is its appearance. Compared to CPS-U, CPS-C facilitates releasing of higher springiness values. The moderate increase, obtained for this TPA-component, most often leads to an increase in chewing during product consumption and depending on the user, can cause approval. It is due to the fact that wide range of cooked sausage consumers associate these products with the typical „popping“ sound, released by biting (Bourne, 2002). That sound and the better expressed product springiness, mainly as a result of higher

protein content, are considered by many consumers as some of most important cooked sausage characteristics. Consumers use these indicators to carry out personal quality control; "fluffy" cooked sausage texture indicates low quality of meat, used for production (Klettner, 1988).

Other texture profile component is adhesiveness. This parameter express how strong the measured sample adheres to analyzer's working body, respectively human teeth, during withdrawal after first compression. Best way of giving physical expression to this parameter represents the determination of withdrawal work.

After conduction of analysis it was found that the differences between samples are very small. Control and experimental samples values are not statistically different, except that of CPC1.5, which is the highest obtained – 0.63 N.mm ($P < 0.05$). Release of higher adhesiveness values would be contributed by the presence of factors, which could be able to facilitate probe adhesion to analyzer's working part. Better expressed adhesiveness could be observed by the presence of thin water layer or small droplets on sample surface, i.e. higher moisture content of sample surface. The moisture and presented salts would promote protein and/or hydrocolloid solubility. The result of such interaction would be formation of sticky film on product surface, which after contact with analyzer's working body, would lead to an increase of the work required for adhesion forces overcoming. Possible reason for the established high value of CPC1.5 could be protein matrix discontinuity and the resulting from these disadvantages, regarding to water and fat immobilization. Pereira et al. (2011) conducted analyses with structureless cooked sausages and reached similar conclusion.

Other sausage texture profile component is cohesiveness. Aim of this indicator is giving information about how measured sample withstands second compression, respectively biting, compared to its behavior after first compression. The parameter is dimensionless and its values vary from 0 to 1. Cohesive products are considered to be those whose structural integrity is responsible for their withstanding in case of exposition to external stress – compression or tensile. These products have the ability to adhere to itself under some compressive or tensile stress (Bourne, 2002).

Conducted analysis indicates that the addition of collagen preparations reveals significant influence on cohesiveness values. According to data, presented in Table 2, samples produced with collagen preparation addition in quantity of ≥ 25.00 g.kg^{-1} exhibit highest values, which are in the range 0.62-0.67. Taking into account additives chemical composition, in particular their high protein content ($\geq$900.00 g.kg^{-1}), it could be argued that observed variations, in regard to samples cohesiveness values, are directly related to preparations protein content. The ability of cohesive products to adhere to themselves should be directly related to the methods through which these form structure, capable of withstanding external stress. Apart from type of external stress – compression or tensile, cohesive products should reveal high degree of organization and structure, facilitating high number of interactions between structure components. The aforesaid, as well as the high cohesiveness values obtained in aforementioned samples, give ground to assert that addition of collagen preparations in production process of structureless cooked sausages type "krenvirsh" lead to increase of parameter values. This is due to the good expressed ability of collagen proteins in regard to bond formation in between, as well with other proteins, presented in meat batter. Reported by Cofrades et al. (1997); Pietrasik (1999); Pietrasik and Duda (2000) cohesiveness values increase, as a result of fat content reducing, confirms the findings of the conducted analyses. Opposite to the established relation, Colmenero et al. (1995) suggested that there is not statistical confirmation supporting this mutual influence. These facts give them reason to conclude that there is no evidence regarding to existence of relation between samples fat content and observed cohesiveness values.

Addition of 15.00 g.kg^{-1} CPS-C results in formation of cohesiveness value of 0.60, established in sample CPC1.5; this result differs statistically from those of control samples and CPU1.5. The established influence in regard to sausage characteristics, result of the addition of same amount CPS-U, is associated with slight increase of parameter values, however established increase is not enough to obtain statistical difference between values of control samples and CPU1.5. This confirms the better expressed influence of CPS-C, compared to that of CPS-U, which is strongly emphasized at low concentrations.

From consumer viewpoint high values of meat product cohesiveness could have negative impact on the sensory evaluation. Considering that aim of TPA-determination is obtaining information about product behavior during chew process in oral cavity, it is reasonable to say that necessity of many chews for achievement of easy-to-swallow texture, make high cohesiveness values unrepresentative for cooked sausages group. Obtaining of too low cohesiveness values is also an indicator of altered product structure. "Crumbliness" and its typical low cohesiveness values, is associated with pronounced product disintegration, result of only few chews. Such texture characteristics are typical for low quality meat products or for such produced with strongly expressed structure defects, and often lead to consumer disappointment in regard to expected and presented sausage quality characteristics (Resurreccion, 2004).

Data obtained for above mentioned TPA-components allows the introduction of new one – chewiness. Its numeri-

cal value, expressed as product of hardness, cohesiveness and springiness values, is used as a parameter that provides information about the work, required for sample chewing (Bourne, 2002). Due to the presence of only one dimensional parameter – hardness (N), chewiness will be expressed with same dimension. This parameter provides an overview of examined structural and mechanical properties of the product, and information about its possible behavior when consumed.

According to the interpretation of conducted analyses, it becomes clear that CPC3.5-sausages consumption requires most energy for chewing. The observed high parameter value, 50.02 N ($P < 0.05$), is expected, considering the release of highest values, obtained for all three components, constituting chewiness product. Reasons, underlying established results, are the same, which had leading role in regard to previously established values for sample hardness, springiness and cohesiveness. The factors associated with their occurrence and influence on texture profile are discussed and presented in the part, related to the three mentioned indicators.

The next highest chewiness values were observed in samples CPC2.5 and CPU3.5, respectively 43.06 and 41.11 N. They do not differ statistically, which means that variations in regard to parameter values in case of 25.00 g.kg^{-1} CPS-C-usage, are identical to these observed when 35.00 g.kg^{-1} CPS-U were used. Other samples values confirmed the established trend, according to which there is value increase in case of CPS-C-usage, compared to usage of same amount CPS-U. Variations between control samples, on the one hand, and samples CPC1.5 and CPU1.5, on the other hand, are the smallest observed. Their moderate rate of increase, compared to that one established in other samples, facilitates producing a product, which displays all typical characteristics of this sausage type. Lower chewiness values obtained in CPU1.5, compared to these in CPC1.5, indicate texture profile improvement and avoidance of the disadvantageous effects, resulting of excessive modification of certain texture parameters, including chewiness, when collagen preparation addition is used (Sielaff, 1996). Established chewiness variations, whose numerical expression is the increase of force (N), in the samples produced with addition in an amount of ≥25.00 g.kg^{-1}, lead to formation of sausages with significant texture characteristics changes.

Microbiological results

Figure 2 and Figure 3 present results from the microbiological analysis of structureless cooked sausage type "krenvirsh" conducted on 1st and 4th day of their refrigerated storage.

Data obtained from microbiological analysis, which was conducted on the 1st day refrigerated storage, show close parameter values for all treatments. In terms of total plate count, samples CPC3.5 and CPU3.5 release lowest values although none of them indicates statistically significant difference. Average amount of aerobic mesophilic microorganisms is 2.43 log cfu.g^{-1}. The effects of nitrite and conducted heat treatment were favorable in terms of total plate count decrease to levels which are described as normal for cooked sausages (Borch et al., 1988; Pegg and Shahidi, 2004). It was established that collagen preparation addition does not lead to an extra microbial contamination of sausages.

The established amount of enterococci, presented in produced sausages, is associated with thermoresistant species, which have the ability to survive the heat treatment, they constitute a considerable part of the residual microflora. Their content is higher in control samples – 1.70 log cfu.g^{-1}

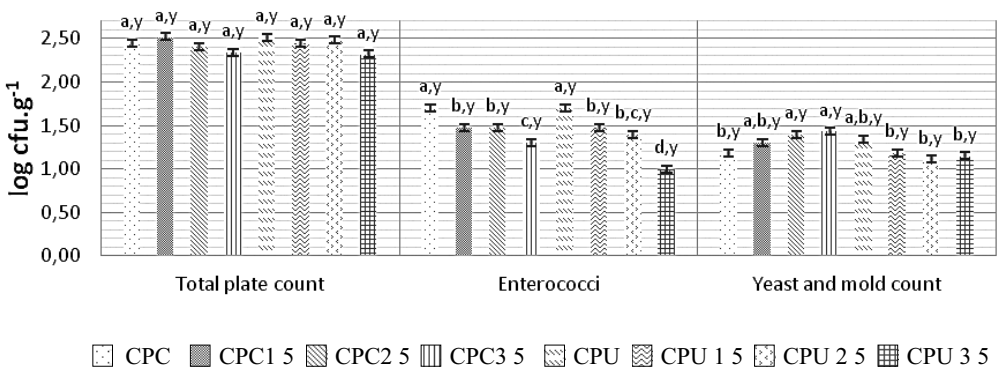

Fig. 2 Microbiological characteristics of structureless cooked sausage type "krenvirsh", 1st day refrigerated storage

a-b – means with same superscripts are not significantly different ($P > 0.05$)

z-y – means of same probe, obtained on 24th hour and 4th day, with same superscripts are not significantly different ($P > 0.05$)

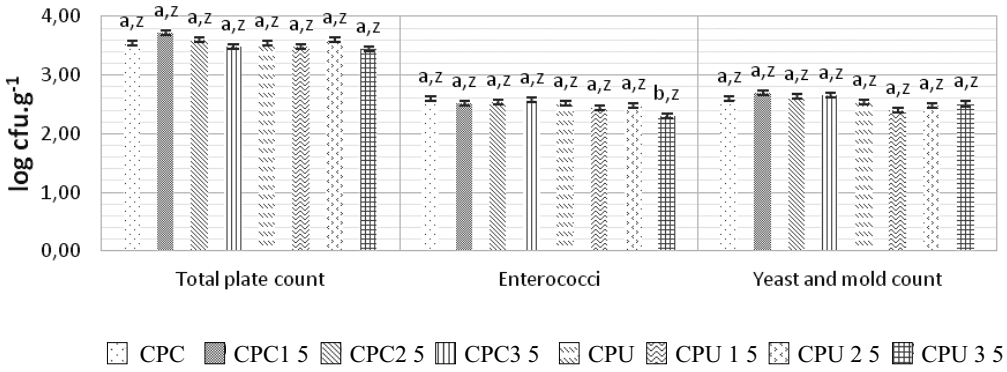

Fig. 3 Microbiological characteristics of structureless cooked sausage type "krenvirsh", 4th day refrigerated storage

a-b – means with same superscripts are not significantly different ($P > 0.05$)

z-y – means of same probe, obtained on 24th hour and 4th day, with same superscripts are not significantly different ($P > 0.05$)

($P < 0.05$). This result is in conformity with the data established for samples water activity values, according to which exactly control samples release highest values ($P < 0.05$). No significant difference was established regarding to sausages amount of yeasts and molds ($P > 0.05$). The average value for all samples – 1.26 log cfu.g^{-1} is higher than expected, considering the conducted heat treatment. Stiebing (1984); Sachindra et al. (2005) reported lower parameter value obtained in structureless cooked sausages. The higher amount of yeasts and molds is probably due to product contamination after producing.

By means of experimental data, obtained from microbiological analyzes, the absence of sanitary-indicative microorganisms from the group of coliforms was established in all samples, in both 1st and 4th day of refrigeration. The samples were tested for presence of pathogens, serving as Food safety criteria: – *Listeria monocytogenes* and *Salmonella ssp.* (Regulation EU 2073/2005, Regulation EU 1441/2007). Such have not been found in 25 g-samples, taken from all treatment.

Results obtained on last day of products shelf life indicate increase of microorganism count in all examined groups during storage phase. This trend remains valid for all samples, as established values on 1st and 4th day differ significantly ($P > 0.05$).

The trend of releasing statistically significant differences between samples, in terms of total mesophilic microorganisms count, remains valid also on the fourth day; the established average value is 3.55 log cfu.g^{-1}. Important parameters of produced sausage type, like water activity, which experimentally obtained values vary in region 0.975-0.980, afford favorable conditions during refrigerated storage for growth of microorganisms, which have remained unaffected during heat treatment or those which contaminate product after its production.

The amount of enterococci also increases and reaches its maximum value of 2.60 log cfu.g^{-1}, obtained in sample CPC. The rapid growth of microorganisms, associated with this parameter, also did not result in releasing of statistically significant differences between the samples in terms of their average values; a single exception was observed in sample CPU3.5, which displays the lowest examined value – 2.30 log cfu.g^{-1} ($P > 0.05$). This statistically significant parameter value is likely, as in the first day of storage, due to the low water activity of the sample ($P > 0.05$). Yeasts and molds number also registered an increase, as examined values for individual samples remained not statistically significant ($P > 0.05$).

As opposed to the pH-decrease obtained in cooked sausage during refrigerated storage, as result of production of lactic acid, acetic acid and fumaric acid, which are products related to the growth of lactic acid bacteria (Pérez-Chabela et al., 2008), conducted analyses indicate slightly pH-increase during storage period. Probably this occurrence can be related to the established higher amount of molds, having the ability to produce alkaline substances, as well as to the limited growth of product residual microflora, including lactic acid bacteria and enterococci.

Conclusion

The conducted experiments undoubtedly confirm the influence of both used collagen preparations on the examined parameters. Addition of CPS-C results in higher values of

pH, while CPS-U has a greater effect on water activity reduction. Collagen preparations exert most significant influence on products texture profile, but their use in concentrations of 25.00 and 35.00 $g.kg^{-1}$ was found to be impractical, due to the significant modification of the TPA-components. The use of collagen preparations caused a slight modification of sausages microbiological characteristics. The effect of addition 35.00 $g.kg^{-1}$ CPS-U can be referred as more significant due to the established statistically significant reduction of *Enterobacteriaceae* and yeasts and molds count. Obtained results allow concluding that 15.00 $g.kg^{-1}$ collagen preparations addition has a beneficial effect on sausages production; it should be noted that better results are found when CPS-U was used.

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Received June, 27, 2016; accepted for printing March, 10, 2017