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EFFECT OF TYPE AND LEVEL OF COLLAGEN SUPPLEMENTS ON MORPHOLOGICAL CHARACTERISTICS OF COOKED SAUSAGES

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Abstract

Despite the undoubtedly proven gel-forming properties of collagen proteins, there are different views in the scientific literature about their use in order to stabilize the raw and heat-treated meat mass in the production of structureless cooked sausages. The aim of this research was to investigate the morphological characteristics of nonstructural cooked sausage produced with two different collagen supplements (CPS-C - commercial one; and CPS-U - laboratory produced from pork skins by mechanical treatment) as well as to establish changes in sensory characteristics.

In the study, six experimental groups of cooked homogeneous sausages were produced with the same basic ingredients, but with different amounts (15; 25, and 35 g x kg^{-1}) of added type of collagen supplements (CPS-C and CPS-U). Sensory evaluation was performed in order to determine general texture acceptability of the type and amount of collagen supplementation levels in the final products. It was used a five-point scale to evaluate four major attributes of indicators, multiplying the score for each of them by a corresponding factor of significance. The total sensory value of the test sample was obtained as a sum of the multiplied estimates of the individual indicators divided by 10. For assessment of the morphological changes, fixed, dehydrated and stained with hematoxylin - eosin cuts of sausages were performed and observed by light microscopy with magnification x 400.

The morphological analysis revealed a great influence on products structure by the type and quantity of collagen supplements used. The addition of 15 g x kg^{-1} CPS-U had a positive effect on structuring process of the protein matrix, immobilizing the water and oil phase as well as reducing the effect of the thermal induced shrinkage of the filling mass. It was observed that the most acceptable texture was established in the sample with 15 g x kg^{-1} collagen supplementations, while concentrations ≥ 25 g x kg^{-1} were associated with not typical and harder consistency.

The addition of 15 g x kg^{-1} CPS-U has a positive effect, resulting in formation of much more stable protein matrix and immobilization of water and fat phase. Collagen concentrations ≥ 25 g x kg^{-1} were not appropriate for structureless cooked sausages.

***Key words**: Collagen supplement, Protein matrix, Protein aggregates, Cooked sausages, Morphological characteristics.*

1. Introduction

The use of collagen preparations is a common practice in the manufacture of cooked sausages. This can be attributed to their broad-spectrum influence on important technological and qualitative parameters of the finished product [1, 2]. A favourable effect related to the water retention, the oil phase immobilisation and the improvement of the structural and mechanical properties has been established [3, 4, and 5], together with a negative effect resulting from significant deviations in the texture and organoleptic characteristics of the

product [6, 7, and 8]. Regardless of the positive or negative character of the changes, the similarity between them lies in the fact that they result from the direct participation of collagen proteins in the processes of formation of the morphological characteristics of the sausages produced. The protein nature of the collagen supplements is the reason for their interaction with the protein and fat fractions of the meat raw materials used in the manufacture. As a result, the collagen supplement could be added evenly to the total meat system, or protein aggregates may be formed leading to a disruption of the continuity of the meat "emulsion". A challenge in the use of such preparations is also the pronounced self-organising capacity of the collagen fraction which facilitates the formation of collagen complexes in the meat mixture and unstable protein films around the fat globules [9].

Pereira *et al.*, [10], cookedsausagequalitycharacteristicswereinvestigatedusingthecentralcompositerotatabledesignofresponsesurfacemethodology (RSM reported a favourable effect of collagen supplements on the stability of heat-treated meat "emulsion" due to their optimum degree of interaction with the myofibrillar proteins in the muscle tissue. The protein mass formation resulting from the inclusion of additional collagen sources contributes to the formation of a protein network capable of immobilising the liquid phase in the multi-component meat mixture [11]. Contrariwise, experiments with structureless cooked sausages conducted by Jones and Mandigo, [12], and Youssef and Barbut, [9], related the use of an additional collagen source to the formation of too thick and hard protein films around the fat globules which could not retain the fat in them. In opposition to the unequivocal stand that collagen proteins have a direct effect on the morphology of cooked sausages, the assessment of their effect has caused divergent comments in scientific circles.

The aim of the present study was to make a comparative analysis of the morphological changes in cooked sausages of the frankfurter type after addition of two different collagen preparations in three concentrations.

2. Materials and Methods

2.1 Materials

2.1.1 Meat raw materials and supplements

The studies were conducted using structureless cooked sausages of the frankfurter type. A control, i.e. CPC, and six experimental samples were prepared for that purpose. Chilled and boned lean and fat pork having pH 6.15 and pH 6.13 respectively were used as meat raw materials. The flake ice and collagen preparation amounts have been specified in Figure 1.

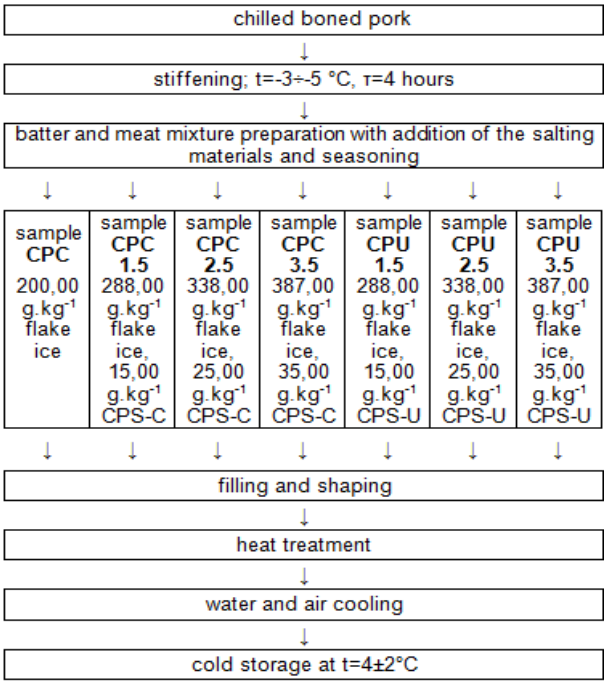

Figure 1. Experimental setup

The experiments were carried out with two types of collagen supplements: 1. Commercial preparation (CPS-C) with protein content ≥ 900 g x kg^{-1}, fat content ≤ 50 g x kg^{-1} and water content ≤ 50 g x kg^{-1} (Scanflavour A/S, Denmark); 2. Preparation (CPS-U), laboratory-made at the Meat and Fish Technology Department, University of Food Technologies, Plovdiv, through mechanical treatment of pork skins supplied by Bony Holding JSC: protein content: 902.7 g x kg^{-1}, fat content: 54.7 g x kg^{-1} and water content: 35 g x kg^{-1}. The sausage recipe included the following: lean pork: 700 g x kg^{-1} and fat pork: 300 g x kg^{-1}. Nitrite salt in 18 g x kg^{-1} amount, 3 g x kg^{-1} of sodium polyphosphate, 0.2 g x kg^{-1} of ascorbic acid, 3 g x kg^{-1} of white pepper, 1 g x kg^{-1} of red pepper, 0.5 g x kg^{-1} of nutmeg, 0.2 g x kg^{-1} of ginger, and 0.2 g x kg^{-1} of cumin were also added to the raw meat mixture. The sausages were manufactured at the training and production facility of the Meat and Fish Technology Department, University of Food Technologies, Plovdiv. The meat was chopped, placed in a cutter and processed together with the salting materials until fine homogeneous mass. The flake ice and collagen supplements were added during cutting. The meat mixture so prepared was stuffed into synthetic polyamide casings and subjected to heat treatment under the following conditions: 1). 55 ^{0}C temperature in the chamber for 10 min. 2). 65 ^{0}C temperature in the chamber for 15 min. 3). 75 ^{0}C temperature in the chamber for 15 min. 4). 80 ^{0}C temperature in the chamber until 72 ^{0}C temperature was reached in the sausage core. The heat-treated sausages were cooled in running water until their temperature was equal to the ambient temperature, then placed in cold storage at t = 2 ± 2 ^{0}C.

2.2 Methods

2.2.1 Morphological analysis

For the purposes of the morphological analysis, cubes with a side length of 1 cm were cut out of the sausages and placed in formalin for fixation. After dehydration in ethyl alcohol with increasing concentrations and double treatment with xylol, the samples were incorporated into paraffin blocks. Using a microtome, 5 μm thick cuts were made in the samples and stained with hematoxylin-eosin according to Mantis *et al.*, [13]. Using an Olympus BX41TF light microscope, Japan, equipped with an Olympus SC30 digital camera, light microscopic observations were made at 400 × magnification.

2.2.2 Sensory analysis

The analysis was made in compliance with the methodology developed by the German Agricultural Union (Deutsche Landwirtschafts-Gesellschaft). The sausages were studied on the 2nd day of their cold storage, and 30 min. before the analysis they were taken out and left at room temperature. The product evaluation was made by completing a tasting card. The main evaluation indicators in it were classified into 4 main categories: 1. Appearance; 2. Cut surface, colour, colour stability and structure; 3. Consistency; 4. Taste and smell. Product was evaluated along a five-point scale, the maximum score for each indicator being 5, multiplied by a corresponding factor of significance: for appearance: x1; for cut surface, colour, colour stability and structure: x3; for consistency: x2; for taste and smell: x4. Reduction in the score given to a certain indicator was based on common flaws in this group of meat products selected from a list in the tasting card. The minimum score needed to cover the minimum criteria for each of the evaluated categories was 3. Sum of the multiplied estimates for the individual indicators was divided by 10. Value thus obtained was the total score for the product tested.

2.2.3 Mathematical and statistical processing of the data

Two-way ANOVA was applied to the assessment of the effect of the collagen preparation type used (Factor I) and its quantity (Factor II) on the sensory evaluations of the ready-to eat products. Tukey's test was applied for multiple comparisons between all mean value pairs. All calculations were made at confidence level $\alpha = 0.05$. The statistical procedures were performed using the Microsoft Excel 5.0 software and the Statgraphics 16 program.

3. Results and Discussion

The images obtained from the morphological analyses made have been presented in Figure 2 - 4. In the CPC control sample (Figure 2a, 2b), a protein matrix formed by the interactions between the dissolved protein fractions played the role of a main structural element. The image in Figure 2a shows that within this structure, there were optically denser zones, irregular in shape and size, made from muscle fragments, cell organelle residues and sarcoplasmic reticulum. The preparation of the meat mixture was accompanied by the incorporation of air dispersed in the meat system in the form of air bubbles of different sizes (Figures 2a, 2b).

Another basic element in the morphological picture is the dispersed fat in the form of droplets. In the CPC, there were fat globules with sizes which significantly exceeded those of the most common ones in the other samples. Their shape was also significantly changed and as a result, they had lost their ovalness to a greater or lesser degree (Figure 2b).

The formation of big fat depots most probably resulted from the flocculation of several closely located small fat droplets which was later transformed into coalescence [14]. This phenomenon has a strong

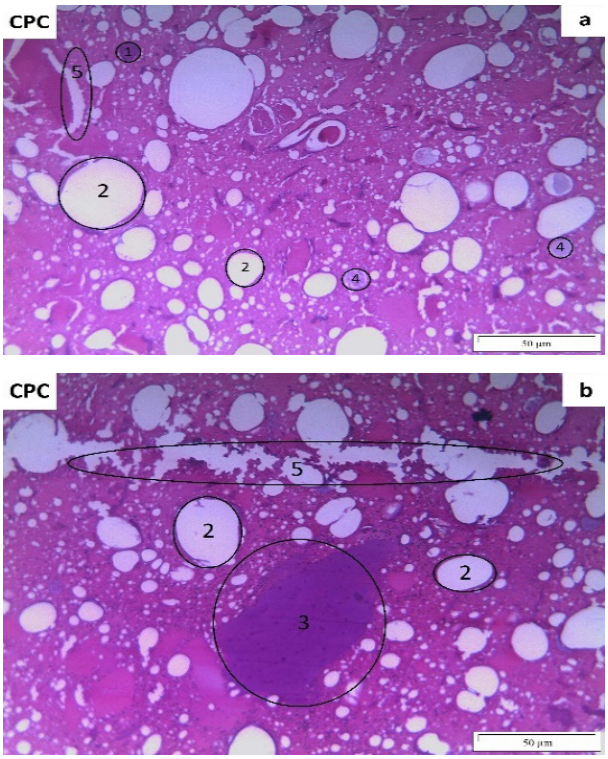

Figure 2. Light microscopic images of sample CPC magnification 400×; 1. Higher optical density zone; 2. Air space; 3. Fat globule with distorted shape and/or surface accumulations; 4. Fat globule with intact shape; 5. Matrix disruption as a result of the formation of a system of "channels"

negative effect since it may lead to the formation of "air pockets" in the finished product which will be filled with water or fat. This may be attributed to the changes occurring during the heat treatment of the meat mixture. With the increase in temperature, fine protein network of the raw meat mixture is subjected to increasingly stronger thermomechanical contractions. Appearance of a large number of links among the protein particles is accompanied by their movement close to one another, and this causes partial shrinkage of the protein network. Simultaneously occurring processes of heat-induced contraction of the matrix and expansion of the fat matter is the reason for the fat globule and water migration accompanied by the formation of "channels" in the structure of the finished product, which can be seen in Figure 2b. They disrupt the intactness of the protein network and that leads to destabilisation of the meat "emulsion" [15, 16].

Samples made with the inclusion of a protein additive exhibited differences from the control sample with regard to their morphological characteristics. Samples CPC1.5 (Figure 3a, 3b) and CPU1.5 (Figure 4a, 4b) were similar in structure, which was probably due to the same quantity of preparation used. In both samples, the incorporated air amount increased, but unlike the CPC, it was dispersed in them in the form of air bubbles of reduced size. Apart from the difference from the control sample, a difference was also observed between the samples themselves after the addition of 15 g x kg^{-1} of preparation. Figures 4a and 4b show the spaces occupied by air in sample CPU1.5. Their round shape is better preserved and they are smaller in size than those in CPC1.5 (Figure 3a, 3b). The reason for this could be found in the better organisation of the protein matrix. The microscopic image of CPC1.5 (Figure 3b) shows clearly several large air spaces formed by the

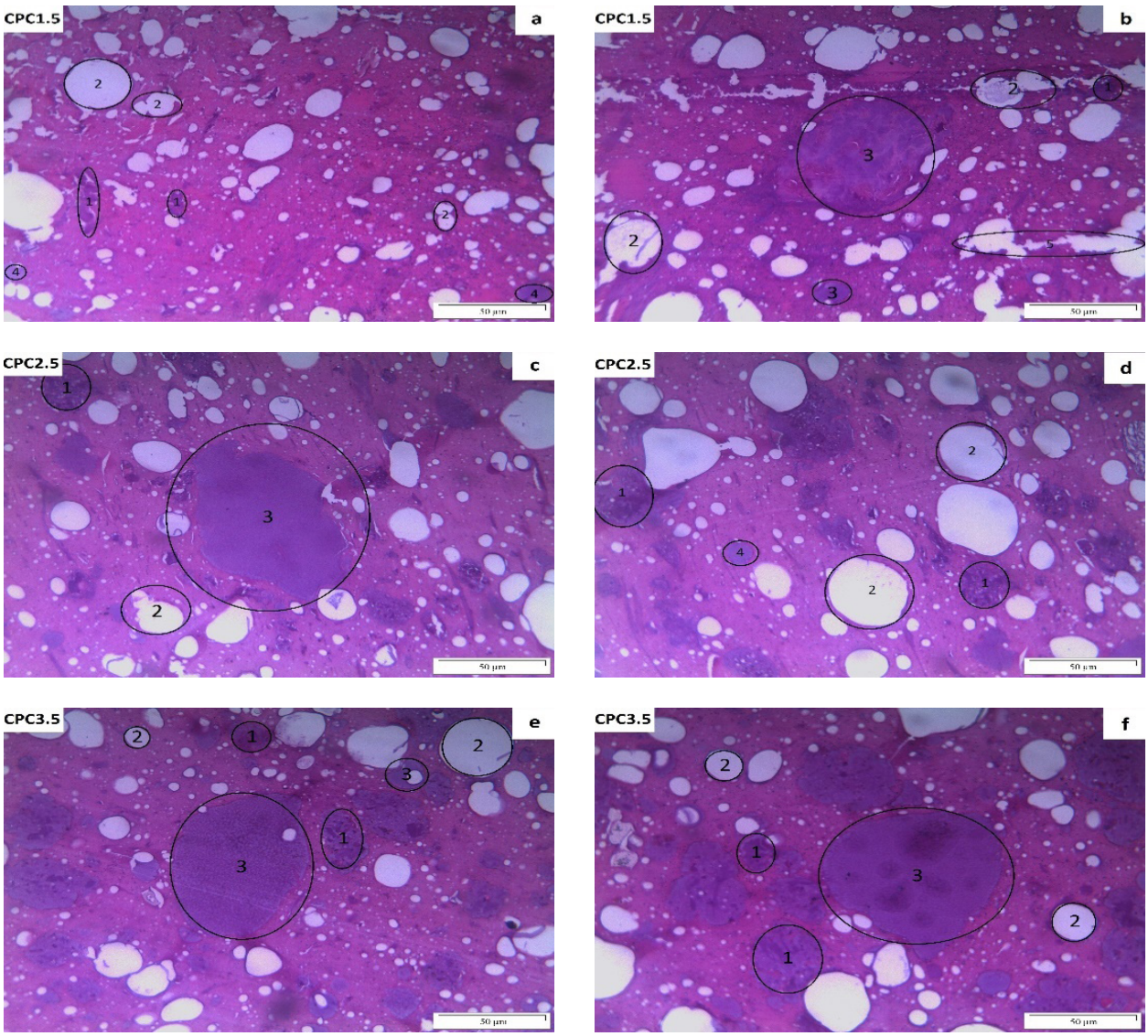

Figure 3. Light microscopic images of samples CPC1.5 - a, b; CPC2.5 – c, d; CPC3.5 – e, f magnification 400×; 1. Higher optical density zone; 2. Air space; 3. Fat globule with distorted shape and/or surface accumulations; 4. Fat globule with intact shape; 5. Matrix disruption as a result of the formation of a system of "channels"

merging of two or more smaller ones. A possible reason for their formation is the increased protein content of the two samples in comparison with the control. The larger protein quantity may cause aggregation processes which have a negative effect on the organisation and continuity of the meat matrix owing to the uneven distribution of the protein fraction [17].

The CPU1.5 images (Figure 4a, 4b) illustrate the advantage of the sample over the CPC1.5 consisting in the better distribution of the collagen preparation, which in turn reduced the possibility of protein aggregate formation. Excessive aggregation causes a reduction in the water-retaining capacity of the meat "emulsion", increase in heat losses and conversion of the protein matrix into a coarse-structured network as a result of the formation of "channels" in the product [15, 18]. The good distribution of the collagen preparation in the CPU1.5 sample as seen in Figure 4a and 4b not only eliminated the negative effect of the protein aggregation but also contributed to a positive effect of the increased protein content. The additional protein quantity led to a moderate increase in the protein interactions which contributed to the formation of a denser matrix characterised by a higher immobilisation degree of the water and fat matter. Furthermore, it is worth mentioning that there were no morphological changes caused by aggregation processes [9]. Most of the fat globules in the CPU1.5 sample had an intact spherical shape and size < 20 µm, which allowed this system to be classified as real emulsion [19, 20]. Figure 4a and 4b show clearly that with few exceptions, the fat globule surface was even and smooth, without any excessive deposits of agglomerations of different components of the meat system. The accumulation of

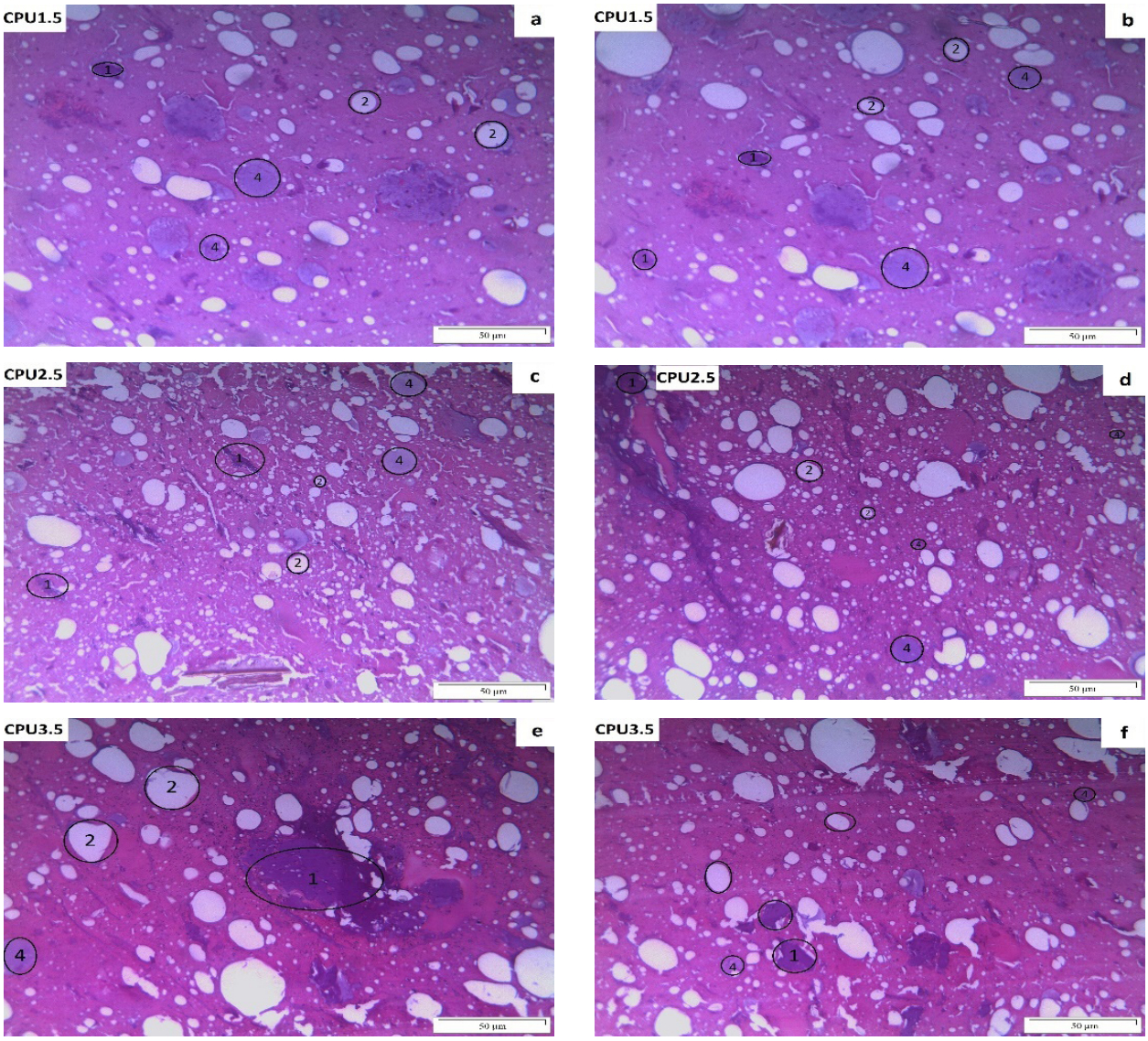

Figure 4. Light microscopic images of samples CPU1.5 - a, b; CPU2.5 – c, d; CPU3.5 – e, f magnification 400×; 1. Higher optical density zone; 2. Air space; 3. Fat globule with distorted shape and/or surface accumulations; 4. Fat globule with intact shape; 5. Matrix disruption as a result of the formation of a system of "channels"

myofibrillar particles, filaments, protein molecules, etc. led to the formation of a thicker film which became considerably harder during heat treatment. This hardening, together with the significantly reduced elasticity and the film's inability to resist the tension caused by the thermal expansion of the fat matter, is a prerequisite for the cracking of the film and the leakage of fat out of the globule [21, 22]. Perhaps this was the reason for the formation of a fat globule with strongly disrupted protein film and leaked-out fat matter in sample CPC1.5 presented in Figure 3b. Figure 3c, 3d and Figure 4c, 4d show the microscopic images of samples CPC2.5 and CPU2.5, respectively. Unlike the previous samples with the same protein additive quantity, these exhibited a pronounced difference. Sample CPC2.5 showed the presence of air spaces of medium and big size (> 50 μm) incorporated into the finished product structure (Figure 3d). In spite of the fact that the "channel" system which disrupted the intactness of the matrix cannot be seen in the images, the size and quantity of the air spaces were a clear indication of a matrix in which the air and fat matter immobilisation were not completed prior to the heat treatment process.

The excessive incorporation of air reduced the self-organising capacity of the meat mixture components. In addition, the air bubbles had the ability to attract and then use the available protein fraction in order to form a protective film around themselves [14]. These negative effects were enhanced by the uneven distribution of the protein fraction and other muscle tissue elements seen in Figure 3c and 3d. Unlike the previous samples, the protein aggregation occurred to a larger degree in these ones. There were aggregates of optically higher density, a large number of them deposited on the fat globule surface and serving as a barrier between them and the surrounding protein matrix. Having in mind that the average fat cell size is 70 - 80 μm [22], it can be said that the large fat globules appeared during the meat mixture formation or at the beginning of the heat treatment as a result of flocculation. Regardless of the large size of some of them, the protein fraction concentration on their surface which acted as a protective film prevented the destruction of the meat "dough" structure. The situation with sample CPU2.5 was different from the one described so far. The images in Figure 4c and 4d demonstrate a well-organised and structured protein matrix, in which the considerable amount of incorporated air is clearly visible. An interesting point to be made after the comparison with CPC2.5 (Figure 3c, 3d) is the difference in the number and size of air spaces. In spite of their large number and proximity, no big spaces were formed as a result of the merging of several smaller ones. The reason for the different structure of samples CPC2.5 and CPU2.5 could also be sought in the different structure of the main morphological element, i.e. the protein matrix. The CPS-U included in the meat system assisted to a larger extent the myofibrillar proteins which were the main factor in the water and fat immobilisation.

As far as the fat emulsification is concerned, the CPU2.5 sample also had a significant advantage. In it, the fat matter was in the form of droplets which had preserved their round shape and small sizes after the heat treatment (Figure 4c).

The results of the morphological analyses of samples CPC3.5 and CPU3.5 (Figure 3e, 3f and Figure 4e, 4f) demonstrated similarities between the samples in terms of flaws consisting in the increased area of zones occupied by protein aggregates. The formation of these zones was directly related to the increased protein additive content, its quantity being the highest in these samples. The possibility of interaction between muscle proteins and the connective tissue proteins added, as well as among the connective tissue proteins, was a prerequisite for their easier inclusion in aggregation processes (Figure 3e and Figure 4e). Although disadvantages were observed with both samples, they were more pronounced in CPC3.5 (Figure 3e, 3f). Aggregates disrupted the matrix continuity and homogeneity [16], [17]. A smaller area of the optically denser zones was observed in CPU3.5 (Figure 4e, 4f), and the matrix preserved its original form to a larger extent.

Figure 3e and 3d show that the lipid fraction dispersion in CPC3.5 was in the form of large fat globules > 50 μm in size. Their irregular shape suggested that their original appearance had been modified as a result of deformations caused by the shrinkage of the matrix and expansion of the fat matter in them. Their shape was a clear indication of the migration which had occurred during the heat treatment, consequently making it impossible to preserve the characteristic round shape of the fat droplets in the raw batter. Accumulation of protein mass, not well-distributed within the matrix, on their surface also contributed to their predominantly large size. Furthermore, the images showed fat globules < 50 μm in size, which nevertheless did not have the characteristic round/oval shape. The accumulation of protein mass on them was a factor in the prevention of the flocculation process but the irregular protein adsorption had a negative effect on the preservation of their shape. Distribution of incorporated air in sample CPU3.5 (Figure 4e, 4f) was in the form of smaller air spaces. No big formations were observed in CPC3.5 either, but the considerable presence of protein aggregates reduced the quantity of the air incorporated in the system (Figure 3e, 3f).

Sensory characteristics are considered a key factor in the evaluation of the consumer value of products since consumers choose products which have a certain sensory profile [23]. The collagen supplement and its concentration affected the sensory characteristics of the finished product (Table 1).

Table 1. Sensory evaluation of the ready-to-eat sausages

Sample	Appearance	Cut surface, colour, and structure	Consistency	Taste and smell	Total score
CPC	4,63 ± 0,74^{a}	12,38 ± 2,50^{a}	8,25 ± 1,98^{a,c,b}	18,50 ± 2,98^{a}	4,38 ± 0,82^{a}
CPC1.5	4,75 ± 0,46^{a}	11,63 ± 1,92^{a,b}	8,75 ± 1,49^{a,b}	17,50 ± 2,98^{a,b}	4,26 ± 0,37^{a}
CPC2.5	4,75 ± 0,46^{a}	10,88 ± 2,75^{a,b}	5,00 ± 1,51^{e}	13,63 ± 2,72^{b,c}	3,43 ± 0,34^{b}
CPC3.5	4,75 ± 0,46^{a}	9,75 ± 2,12^{a,b}	6,00 ± 1,85^{c,e,d}	12,25 ± 2,66^{c}	3,28 ± 0,41^{b}
CPU1.5	4,75 ± 0,46^{a}	13,13 ± 2,23^{a}	9,75 ± 0,71^{a}	18,00 ± 2,14^{a}	4,56 ± 0,55^{a}
CPU2.5	4,75 ± 0,46^{a}	9,75 ± 3,11^{a,b}	5,75 ± 1,67^{d,e}	9,50 ± 2,07^{c}	2,98 ± 0,73^{b}
CPU3.5	4,50 ± 0,53^{a}	7,88 ± 2,23^{b}	6,75 ± 1,49^{b,d,e}	11,50 ± 2,56^{c}	3,06 ± 0,68^{b}

Legend: $^{a-e}$ - values within same column bearing a common superscript did not differ statistically ($P < 0.05$).

No differences ($P > 0.05$) were observed between the samples with regard to the product appearance indicator. There were no signs of migrated fat, which was indicative of good emulsion stability in all samples. When the casing of sample CPC3.5 was removed, accumulation of jelly-like matter was observed at the end of the sausage, in close proximity to the sausage twist. This phenomenon was caused by a peculiarity in the microstructure of the meat mixture which had undergone heat treatment. The larger intermediate spaces between the microflakes in the coagulated meat mixture could not retain the whole hydrate water quantity released during the protein mass coagulation and that led to its accumulation at the sausage ends [14]. Cut surface of the samples was characterised by homogeneity since no particles had sizes different from the total mass. This is an essential condition in the production of this type of structureless sausages. Unfavourable changes in colour were observed in the samples to which collagen preparation was added in ≥ 25 g x kg^{-1} quantity. The impaired colour characteristics consisted in fading of the product colour and a less pronounced red colour. Samples CPC1.5 and CPU1.5 with minimum collagen addition and the control sample received higher scores on this indicator.

The most significant differences between the samples studied were observed with regard to the consistency indicator. The control sample, sample CPC1.5 and sample CPU1.5 scored high, the highest score of 9.75 having been reported for CPU1.5. For the samples to which collagen preparation was added in ≥ 25 g x kg^{-1} quantity, the consistency was described as "too hard", "untypical of the product". This flaw was a direct result of the increased protein additive quantity, which led to significant deviations from the otherwise positive effect of collagen preparations on the texture of cooked sausages [10]. With regard to the "taste and smell" indicator, scores that were the closest to those expected from the tasters were reported for the control sample, for sample CPC1.5 and sample CPU1.5. Considerably lower scores were given to samples CPC2.5, CPC3.5, CPU2.5 and CPU3.5. Unlike the use of other protein preparations, e.g. soy [1], no off-flavour was established as a result of the use of the protein additive. The importance of muscle and fat tissue in the formation of the flavour bouquet of meat products [24, 25, and 26] and the significantly larger quantity of water included in samples CPC2.5, CPC3.5, CPU2.5 and CPU3.5 reduced the intensity of the taste and smell, making them untypical of a structureless cooked sausage of the frankfurter type. According to the total scores of the experiment made, the highest organoleptic score was given to samples CPC, CPC1.5 and CPU1.5, which differed from the rest of the samples ($P < 0.05$). The addition of 15 g x kg^{-1} of university collagen preparation during the manufacture of structureless cooked sausage of the frankfurter type did not result in defects in its sensory characteristics and had a favourable effect on the finished product by improving its morphological structure and consistency.

4. Conclusions

- Major factors in the formation of the morphological characteristics of the sausages studied were both the concentration and the type of the protein preparation. The inclusion of collagen preparations in large concentrations, i.e. 25.00 g.kg^{-1} and 35.00 g.kg^{-1}, was accompanied by the occurrence of flaws in the protein matrix. The existence of agglomerations of aggregated proteins and large fat droplets resulting from flocculation and coalescence processes, as well as the incorporation of large air spaces were part of them.

- One advantage of the use of CPS-U was established in the sense that these flaws were less pronounced. A negative effect of collagen preparations expressed as lower sensory scores for the colour and consistency criteria was only established when the preparations were added in ≥25.00 g.kg^{-1} quantities.

- On the basis of the results of the morphological and sensory analyses conducted, it could be assumed that CPS-U could also be widely used in the production of other cooked sausage types. The even distribution of the collagen phase and its inclusion in the protein network of the product leads to the formation of a protein network with the best expressed homogeneity and stable colour.

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