FRM

Foods and Raw Materials, 2020, vol. 8, no. 2

E-ISSN 2310-9599
ISSN 2308-4057

Research Article
Open Access

Check for updates

DOI: http://doi.org/10.21603/2308-4057-2020-2-259-267
Available online at http://jfrm.ru/en

Effects of non-meat proteins on the quality of fermented sausages

Ana Velemir*, Snježana Mandić, Goran Vučić, Danica Savanović

University of Banja Luka, Banja Luka, Bosnia and Herzegovina

* e-mail: ana.velemir@tf.unibl.org

Received February 20, 2020; Accepted in revised form March 05, 2020; Published June 04, 2020

Abstract:
Introduction. Non-meat proteins are widely used in meat processing. In our study, we analyzed the effects of whey and soy protein isolates on the physicochemical and sensory properties of domestic fermented sausage.
Study objects and methods. Five groups of sausages were traditionally fermented under industrial conditions. The sausage group without the additives was labelled the control, while other sausages were manufactured with the addition of 0.5% and 1.5% protein isolates of whey and soybean. Using a quantitative descriptive test, we assessed the sensory characteristics of the sausages and instrumentally determined their color, hardness, water activity (a_w), and pH.
Results and discussion. The proteins added to fermented sausages improved emulsification, texture, as well as water and fat binding capacity, which was confirmed by the results for hardness. Using a 0.5% soy protein isolate resulted in a firmer product. The additives had a minor effect on the color: the samples with the additives had a slightly lower L^* value, and those with a soy protein had higher yellowness (b^*).
Conclusion. Using the additives did not have a significant effect on the chemical composition and overall sensory quality of all tested samples ($P > 0.05$).

Keywords: Meat products, sausages, whey proteins, soy proteins, sensory quality, color, hardness

Please cite this article in press as: Velemir A, Mandić S, Vučić G, Savanović D. Effects of non-meat proteins on the quality of fermented sausages. Foods and Raw Materials. 2020;8(2):259–267. DOI: http://doi.org/10.21603/2308-4057-2020-2-259-267.

INTRODUCTION

Today, there are many different meat products on the market. Domestic fermented sausages belong to a group of dry fermented sausages, which are produced in a traditional way and have desirable sensory properties. Their sensory characteristics depend on various factors including the selection and quality of raw materials and basic ingredients, the formulation of sausage emulsion, the metabolic activity of epiphytic microflora, the physicochemical changes during smoking and drying, the enzymatic decomposition of proteins and fats, the conditions and length of ripening, as well as external factors (temperature, relative humidity, and air circulation) [1].

The quality of fermented sausages, as well as the changes that occur during fermentation, drying, and ripening, depends not only on the basic components of the sausage emulsions, but also on the additives that affect the transition of the emulsion into the fermented product. Spices, additives, flavoring agents, enzymes, sugars, carbohydrates, fibers, and protein products are commonly used in manufacturing meat products.

When using additives, we should preserve the characteristic properties of meat products [1]. Introducing non-meat ingredients into meat products improves their quality and reduces the cost of production. The most commonly used ingredients are dairy products, eggs, plants, and probiotics, which contribute to increased nutritional value, consumer acceptance, and benefits for human health [2, 3].

The first impression about the quality of fermented sausage is based on the visual experience, or the size, shape, color, and gloss of products, either coated or packed. The outer surface of the sausage depends, above all, on the type and quality of coating and the intensity of smoking (type of wood) and drying. Also, the sensory evaluation of sausages includes the cross-section color. According to the generally accepted criteria for sensory properties of fermented sausages, the filling on the cross-section should have the appearance of a mosaic composed of approximately equal pieces of meat (stable and uniform red color) and fat tissue (whitish color). The filling ingredients must be evenly arranged and firmly interconnected, with no visible cavities or cracks in the cross-section [4].

The formation of odor and flavor of fermented sausages depends on the fermentation of carbohydrates, lipolysis and lipid oxidation, on proteolytic processes, as well as the type and quantity of used spices, salt, and additives [5, 6].

Non-meat proteins, such as soybean and whey proteins, are often used to improve the texture of meat products. These ingredients play an important role in changing the functional properties such as emulsifying, water and fat binding capacity, and texture. They are used as additives that can improve yield and potentially reduce the cost of products [7].

The previous works have studied the use of non-meat proteins in cooked and semi-dry sausages, but there are few studies on their effect on fermented sausages.

The main goal of this research was to study the effect of soy and whey protein isolates on the quality of domestic sausages traditionally fermented under industrial conditions. The proteins were added to improve the quality of sausage, rather than as a substitute for meat. Adding soy and whey protein to domestic fermented sausages and modelling their quantitative ratio during product development can improve the quality of the new product and reduce the manufacturing time.

STUDY OBJECTS AND METHODS

Domestic fermented sausages were produced in a traditional way under industrial conditions. The emulsion consisted of mature pork (59.3%) and beef meat (7.6%) of first and second category, pork back fat (28.7%), nitric salt for curing (2.5%), spices (0.3% garlic in granules, 0.4% hot red pepper, 0.4% sweet red pepper, 0.3% ground black pepper), and additives (0.3% glucono-delta-lactone GDL/TARI S 77 and 0.3% MIOCOLOR VS (a homogeneous mixture of antioxidants based on the salt of ascorbic acid, edible organic acids, and dextrose)).

For this study, we made five samples of domestic fermented sausages: the control (without isolates); with 0.5% of whey isolate (Impact Whey isolate, Myprotein, Norwich, UK); with 1.5% of whey isolates; with 0.5% of soy isolate (IZOPROT S, Ireks Aroma, Zagreb, Croatia), and with 1.5% of soy isolate. Duplicate batches were prepared. The weight of each batch was 40 kg.

After grinding and mixing in the cutter, the sausage emulsion was poured into natural coatings (pork intestine) with a diameter ~ 30 mm. The sausages were first tempered (22°C), then smoked (beech wood) for three days (18°C to 20°C), and finally left for fermentation (ripening) at 16°C. The relative humidity gradually decreased from 85% at the beginning to 65% at the end of ripening. Following the ripening stage, the final sausages were vacuum packed and stored in a cooling chamber at 4°C until sampling. Seven randomly selected sausages were taken after the ripening stage and during storage periods (1, 2, 3, and 6 months).

CIE *L**, *a**, *b** color values (*L** – lightness, *a** – redness, *b** – yellowness) were determined with a Konica Minolta CM 2600d camera (Osaka, Japan). The measurements were carried out on a fresh cut of sausage samples. Five measurements were taken on three cross-sections of two sausages from each treatment. The mean of 30 measurements was recorded for each color parameter.

The hardness/softness was determined by a universal texture meter, a TA.XT plus Texture Analyzer (Stable Micro Systems, Godalming, UK). The cutting force was measured by a Warner-Bratzler contact pin (parameters: 25 kg force, 4 mm/s rate, 20 mm distance). The test samples were prepared by using a mold with eight rectangular shapes (1×1 cm, approximately 5 cm) in which the measurements were performed. The mean of 20 measurements was recorded.

The water activity (a_w) was determined by a LabMaster-aw hygrometer (Novasina, Switzerland) at a constant temperature of 25°C. The mean of 5 measurements was recorded.

pH was measured by a digital pH meter (HANNA HI 99161, Cluj-Napoca, Romania) equipped with a combined penetration tip, which had been calibrated with buffer solutions at pH 4 and 7. The mean of 5 measurements was recorded.

Using quantitative descriptive analysis (ISO 6564:1985[I]), we evaluated the sensory properties of sausages (external appearance, cross-section appearance and color, odor, flavor and taste, texture and overall acceptability). Ten panelists (6 females, 4 males, average age of 35) took part in the evaluation. Based on the average value of ratings for individual characteristics, we calculated the overall quality score of the sausages.

Standard methods were used to analyze the chemical quality parameters: water content – ISO 1442:1997[II]; total fat content – ISO 1443:1973[III]; total protein content – ISO 937:1978[IV]; total ash content – ISO 936:1998[V]; total phosphorus content – ISO 13730:1996[VI]; sodium chloride content – ISO 1841-1:1996[VII]; and nitrite content

---

I ISO 6564:1985. Sensory analysis. Methodology. Flavour profile methods. Geneve: International Organization for Standardization; 1985. 8 p.

II ISO 1442:1997. Meat and meat products. Determination of moisture content (Reference method). Geneve: International Organization for Standardization; 1997. 8 p.

III ISO 1443:1973. Meat and meat products. Determination of total fat content. Geneve: International Organization for Standardization; 1973. 4 p.

IV ISO 937:1978. Meat and meat products. Determination of nitrogen content (Reference method). Geneve: International Organization for Standardization; 1978. 3 p.

V ISO 936:1998. Meat and meat products. Determination of total ash. Geneve: International Organization for Standardization; 1998. 10 p.

VI ISO 13730:1996. Meat and meat products. Determination of total phosphorus content. Spectrometric method. Geneve: International Organization for Standardization; 1996. 12 p.

VII ISO 1841-1:1996. Meat and meat products. Determination of chloride content. Part 1: Volhard method. Geneve: International Organization for Standardization; 1996. 6 p.

Table 1 Chemical composition of sausages with protein isolates (average value ± SD)

Parameter	Control	0.5% whey isolate	1.5% whey isolate	0.5% soy isolate	1.5% soy isolate
Moisture, %	21.95 ± 1.44	21.20 ± 1.44	21.80 ± 1.44	21.97 ± 1.28	22.10 ± 1.80
Ash, %	4.92 ± 0.35	5.01 ± 0.41	4.91 ± 0.28	4.99 ± 0.29	5.06 ± 0.36
Fat, %	49.38 ± 3.44	49.55 ± 2.10	49.58 ± 2.67	50.14 ± 1.54	48.55 ± 1.63
Proteins, %	20.17 ± 1.89	20.75 ± 1.44	20.12 ± 1.15	19.83 ± 1.52	20.92 ± 1.21
Fat-proteins ratio	2.44	2.40	2.47	2.47	2.30
Moisture-proteins ratio	1.19	1.10	1.17	1.18	1.13
NaCl, %	4.50 ± 0.17	4.60 ± 0.36	4.57 ± 0.02	4.52 ± 0.27	4.56 ± 0.32
Total phosphates, %	0.47 ± 0.06	0.47 ± 0.04	0.49 ± 0.07	0.49 ± 0.06	0.51 ± 0.06
Nitrites, mg/kg	3.04 ± 1.80	3.42 ± 1.37	2.84 ± 1.03	3.78 ± 1.41	4.22 ± 1.94

Table 2 Water activity and pH values of sausages with protein isolates (average value ± SD)

Storage period, months		Control	0.5% whey isolate	1.5% whey isolate	0.5% soy isolate	1.5% soy isolate
a_w	0	$0.832^{a,A} \pm 0.0005$	$0.807^{b,A} \pm 0.0005$	$0.819^{c,A} \pm 0.0005$	$0.819^{c,A} \pm 0.0005$	$0.823^{d,A} \pm 0.0005$
	1	$0.816^{a,B} \pm 0.0005$	$0.820^{b,B} \pm 0.0005$	$0.826^{b,B} \pm 0.0005$	$0.818^{a,c,A} \pm 0.0005$	$0.821^{b,c,A} \pm 0.0005$
	2	$0.808^{a,C} \pm 0.0005$	$0.822^{b,B} \pm 0.0005$	$0.836^{c,C} \pm 0.0005$	$0.842^{d,B} \pm 0.0005$	$0.834^{e,B} \pm 0.0005$
	3	$0.824^{a,D} \pm 0.0005$	$0.822^{b,B} \pm 0.0005$	$0.830^{c,D} \pm 0.0005$	$0.807^{d,C} \pm 0.0005$	$0.817^{e,C} \pm 0.0005$
	6	$0.823^{a,D} \pm 0.0005$	$0.822^{a,B} \pm 0.0004$	$0.831^{b,D} \pm 0.0004$	$0.807^{c,C} \pm 0.0008$	$0.815^{d,C} \pm 0.0004$
pH	0	$5.34^{a,A} \pm 0.063$	$5.54^{b,c,A} \pm 0.001$	$5.37^{a,A} \pm 0.020$	$5.51^{b,A} \pm 0.014$	$5.64^{c,A} \pm 0.016$
	1	$5.57^{a,c,B,C} \pm 0.020$	$5.45^{b,B} \pm 0.050$	$5.53^{a,b,B} \pm 0.005$	$5.62^{c,B} \pm 0.020$	$5.63^{c,A} \pm 0.030$
	2	$5.70^{a,D} \pm 0.005$	$5.64^{b,C} \pm 0.010$	$5.60^{c,C} \pm 0.012$	$5.59^{c,B} \pm 0.010$	$5.68^{a,A} \pm 0.007$
	3	$5.68^{a,C,D} \pm 0.037$	$5.70^{a,b,C} \pm 0.008$	$5.71^{a,b,D} \pm 0.025$	$5.68^{a,C} \pm 0.010$	$5.77^{b,B} \pm 0.030$
	6	$5.48^{a,b,B} \pm 0.041$	$5.48^{a,b,A,B} \pm 0.010$	$5.50^{a.B} \pm 0.023$	$5.44^{b,D} \pm 0.012$	$5.55^{c,C} \pm 0.012$

a–d values in the same column with different superscripts are significantly different ($P < 0.05$)
A–D values in the same row with different superscripts are significantly different ($P < 0.05$)

– ISO 2918:1975[VIII]. All measurements were carried out in 5 repetitions. All analyses were performed immediately after production (0) and during storage (1, 2, 3, and 6 months).

Statistical analysis. Our results were presented as mean values accompanied with standard deviations. A factorial analysis of variance (ANOVA) was performed using the Statgraphic Plus 5.1 Professional Edition (1994–2001, Statistical Graphics Corporation, USA). The Multiple Range test was used to identify significant ($P < 0.05$) differences between treatments. Repeated measures ANOVA was used to test the differences during storage periods.

RESULTS AND DISCUSSION

The chemical composition of all the samples of domestic fermented sausages is shown as mean values of parameters measured after 0, 1, 2, 3, and 6 months of storage (Table 1).

The moisture content of the sausage samples during the period monitored ranged from 21.2% to 22.1%. However, numerous studies report higher moisture contents for similar products [8–10]. Using coatings with a narrow diameter (about 30 mm), longer ripening or a higher fat content could result in a lower moisture content [11]. The fat content of the final product varied between 48.55% and 50.14%, which depended primarily on the recipe, with similar fat contents reported by numerous other studies [8, 12]. According to our results, soy and whey proteins in concentrations of 0.5 and 1.5% did not have significant effects on the total protein content ($P > 0.05$), which agreed with earlier studies [13, 14]. The difference between the contents of fat and protein was large, due to a high content of fat in the formulation, while the moisture and protein values were almost identical, compared to other data for traditional products [5].

The salt content ranged from 4.5 to 4.6%, and other authors obtained similar or higher values for traditionally fermented sausages [15, 16]. The ash contents ranged from 4.9 to 5.06%, while the use of additives, whey and soy protein isolates, did not have a significant effect on the values studied [17, 18].

The average values of total phosphorus during storage ranged from 0.47 to 0.51%, with no major differences between the samples. This result was quite expectable as the meat protein content, the main source of phosphorus, did not change significantly ($P > 0.05$).

The values of residual nitrite content after production and during storage ranged from 2.84 to 4.22 mg/kg. These data confirm the fact that the nitrites were decomposed during ripening and fermentation, which

[VIII] ISO 2918:1975. Meat and meat products. Determination of nitrite content (Reference method). Geneve: International Organization for Standardization; 1975. 3 p.

Table 3 Hardness of sausages with protein isolates (average value ± SD)

Samples	Storage period, months				
	0	1	2	3	6
Control	$1.43^{a,A} \pm 1.30$	$2.12^{a,B} \pm 1.01$	$2.02^{a,B} \pm 0.67$	$1.98^{a,b,B} \pm 0.61$	$2.05^{a,B} \pm 0.62$
0.5% whey isolate	$1.12^{a,A} \pm 0.63$	$1.66^{a,B} \pm 1.14$	$1.60^{a,A,B} \pm 0.92$	$1.59^{a,A,B} \pm 0.78$	$1.62^{b,B} \pm 0.62$
1.5% whey isolate	$1.14^{a,A} \pm 0.62$	$1.62^{a,B} \pm 1.04$	$1.77^{a,B} \pm 0.85$	$1.81^{a,b,B} \pm 0.71$	$1.84^{a,b,B} \pm 0.59$
0.5% soy isolate	$1.00^{a,A} \pm 0.29$	$3.70^{b,B} \pm 1.64$	$3.41^{b,B} \pm 1.35$	$3.16^{c,B} \pm 1.08$	$3.04^{c,B} \pm 0.79$
1.5% soy isolate	$1.06^{a,A} \pm 0.41$	$2.06^{a,B} \pm 1.16$	$2.02^{a,B} \pm 0.77$	$2.11^{b,B} \pm 0.80$	$2.07^{a,B} \pm 0.59$

a, b values in the same column with different superscripts are significantly different ($P < 0.05$)
A, B values in the same row with different superscripts are significantly different ($P < 0.05$)

was reported by many authors [8, 19]. In the Slavonian sausage of Kulen, the content of nitrite after ripening was 2.93–14.3 mg/kg [20]. As we can see, there were no significant differences ($P > 0.05$) in the chemical composition between the samples.

The degree of reducing the a_w value depends on the composition of sausages, temperature, relative humidity, and the ripening time. During drying and ripening, the concentration of water in the product decreases, followed by dehydration and reduction of a_w [11].

The results of water activity can be seen in Table 2. After production, the a_w values of the analyzed samples ranged from 0.807 to 0.832, which was confirmed by Suvajdžić [16]. Mastanjević received higher values in the study of Slavonian kulen [21]. Operta *et al.* reported that the activity of water in traditional fermented sausages ranged from 0.83 to 0.89 at the end of drying, which was also the case for dry fermented chicken sausages with the addition of corn oil and soybean isolates [22, 23]. During storage, there were noticeable significant differences ($P < 0.05$), with values of a_w ranging from 0.807 to 0.842 (Table 2). Operta *et al.* reported similar results indicating that the products with a soybean protein isolate showed a slight decrease in a_w values during storage [22].

After the production, the pH values of the samples were from 5.34 to 5.64 (Table 2). During storage, they ranged from 5.44 to 5.77, with noticeable significant differences ($P < 0.05$). Many authors cited similar or lower values as a characteristic of fermented sausages [24, 25].

Table 3 shows changes in the samples texture after production and during a six-month storage period. After production, there were no significant differences between the sausages ($P > 0.05$), with hardness ranging from 1.00 to 1.43. Similar values were recorded in other studies as well [1, 26]. Some authors cite higher values [9, 16]. Lee found that the products with the addition of a soybean protein isolate show slightly higher hardness values compared to the control sample [27].

After the first month of storage, there was an increase in hardness, especially in the sample containing 0.5% soy protein isolate compared to other samples ($P < 0.05$). The texture of fermented sausages is related to the fat and salt content, as well as pH [16].

The hardness test showed a noticeable effect of the additives. The samples with a whey protein isolate had a lower cutting force than the control, while the sample with 0.5% soy protein isolate had significantly higher hardness values during the entire storage period ($P < 0.05$).

Priyadarshi pointed out that added soy and whey proteins increased the hardness of cooked pork sausage, while many authors stated the opposite for cooked sausages and burgers [17, 28, 29]. Akesowan found that an amount greater than 2% of soy protein isolate affected the strength of cooked pork sausages [13].

The lightness (L^*) values of the sausage samples are shown in Table 4. As we can see, they were consistent after production, ranging from 49.07 to 50.20. Many studies featured similar values [31, 32]. Kim *et al.* reported higher L^* values, while most authors found significantly lower values, ranging from 30 to 45 [5, 18, 23, 32].

During storage, the L^* values changed significantly ($P < 0.05$) from 40.72 to 50.92, although there was generally a slight decrease. Some studies showed similar results [21, 33]. The decrease in the L^* values was related

Table 4 L^* values of sausages with protein isolates during storage (average ± SD)

Samples	Storage period, months				
	0	1	2	3	6
Control	$49.61^{a,A} \pm 4.26$	$49.79^{a,A} \pm 5.91$	$42.52^{a,b,B} \pm 6.21$	$50.41^{a,A} \pm 5.47$	$46.52^{a,C} \pm 3.09$
0.5% whey isolate	$50.16^{a,A,C} \pm 7.22$	$50.92^{a,A} \pm 6.31$	$42.93^{a,b,B} \pm 6.09$	$44.29^{a,A,C} \pm 5.12$	$46.94^{a,C} \pm 4.89$
1.5% whey isolate	$49.41^{a,A} \pm 4.64$	$48.15^{a,b,A} \pm 4.73$	$44.57^{a,B} \pm 5.05$	$44.36^{b,B} \pm 5.17$	$45.15^{a,B} \pm 3.037$
0.5% soy isolate	$50.20^{a,A} \pm 4.65$	$45.89^{b,B} \pm 4.04$	$42.94^{a,b,C} \pm 3.145$	$43.09^{b,C} \pm 3.91$	$45.65^{a,B} \pm 3.77$
1.5% soy isolate	$49.07^{a,A} \pm 5.18$	$45.54^{b,B} \pm 4.22$	$40.72^{b,C} \pm 5.32$	$44.67^{b,B} \pm 4.32$	$45.83^{a,B} \pm 3.31$

a, b values in the same column with different superscripts are significantly different ($P < 0.05$)
A–C values in the same row with different superscripts are significantly different ($P < 0.05$)

Table 5 a^* values of sausages with protein isolates during storage (average ± SD)

Samples	Storage period, months				
	0	1	2	3	6
Control	$14.50^{a,A}$ ± 2.25	$12.37^{a,B}$ ± 2.88	$16.56^{a,c,C}$ ± 2.45	$12.32^{a,B}$ ± 2.77	$14.85^{a,A}$ ± 2.71
0.5% whey isolate	$12.72^{b,A,B}$ ± 2.82	$12.14^{a,B}$ ± 2.87	$14.53^{b,C}$ ± 2.51	$12.06^{a,B}$ ± 1.81	$14.01^{a,B,C}$ ± 2.15
1.5% whey isolate	$14.15^{a,b,A}$ ± 2.29	$12.12^{a,B}$ ± 2.31	$15.45^{a,b,C}$ ± 2.95	$12.86^{a,b,B}$ ± 2.02	$12.20^{b,B}$ ± 1.91
0.5% soy isolate	$14.30^{a,A}$ ± 2.81	$14.67^{b,A}$ ± 2.18	$17.60^{c,B}$ ± 3.00	$14.58^{c,A}$ ± 2.26	$16.48^{c,B}$ ± 2.05
1.5% soy isolate	$14.24^{a,A}$ ± 2.22	$13.46^{a,b,A}$ ± 2.31	$17.93^{c,B}$ ± 2.83	$14.15^{b,c,A}$ ± 2.58	$14.53^{a,A}$ ± 1.58

a–c values in the same column with different superscripts are significantly different ($P < 0.05$)
A–C values in the same row with different superscripts are significantly different ($P < 0.05$)

to the loss of moisture and also to low fat and high water contents [4, 25].

Škaljac reported that the loss of water from the Petrovačka sausage during aging increased the concentration of myoglobin [11]. On the other hand, the dehydrated muscle tissue absorbed a higher amount of light which resulted in a darker color of the product and decreased L^* values.

In the soy protein samples, the L^* values remained approximately the same or decreased, although some authors had opposite results [13, 14, 23, 34]. Using whey protein also led to lower L^* values [18, 35]. Serdaroglu, however, claimed that milk additives slightly increased L^* values, with similar observations made by Hughes *et al.* [17, 36]. Barbut reported no significant changes caused by whey supplements [37].

The values of redness (a^*) are shown in Table 5. After production, these values ranged from 12.72 to 14.50. This color parameter was significantly different between the samples and during storage ($P < 0.05$). Many authors reported higher values and greater deviations [4, 31]. A reduction of the a^* value was due to a higher amount of lactic acid, which denatured myoglobin, nitrosylmyoglobin, and oxymyoglobin [4]. A lower protein content had the same effect, while a low fat level and a high water content led to increased a^* values.

Serdaroglu and Abdolghafour found no effect of dairy supplements on the a^* value, although some authors reported a decline in this value when using additives, which was confirmed by our study [17, 18, 37]. The use of soy protein resulted in lower a^* values [13, 34].

The values of yellowness (b^*) are shown in Table 6. Immediately after production, they ranged from 12.35 to 14.79 in the 0.5% whey protein and 0.5% soy protein samples, respectively. However, during further storage, this parameter reached 12.49 and 18.00 for the 0.5% whey protein and 0.5% soy protein samples, respectively. Thus, the differences between the samples and during storage were significant ($P < 0.05$). Similar data were reported by other authors [4]. Lower values were given by Skaljac *et al.* for sausages stored under controlled conditions in an industrial chamber [4]. Higher values for vacuum-packed Petrovska sausage were reported by Skaljac *et al.* [38]. The decrease in the b^* values was assumed to be caused by microorganisms that use oxygen during fermentation, thus reducing the amount of a muscle pigment that beneficially affected the b^* value [37]. Another study reported higher fermentation temperature and the addition of autochthonous starter culture as a cause of the decrease [21].

Most authors agree that adding whey and soy protein isolates decreases the b^* values or that they do not change significantly [34, 37]. Abdolghafour reported that the decrease of the b^* value was caused by soy protein, which was confirmed by our results [18]. Hughes *et al.* found that adding whey protein lead to an increase in lightness (L^*) and a decrease in redness (a^*) and yellowness (b^*) [36].

The external appearance of the sausages at the end of production, as well as during the storage period, was satisfactory without any statistically significant differences between the samples ($P > 0.05$). The coat was not separated from the emulsion, deformed or

Table 6 b^* values of sausages with protein isolates during storage (average ± SD)

Samples	Storage period, months				
	0	1	2	3	6
Control	$13.93^{a,b,A,B}$ ± 3.13	$14.20^{a,b,A,B}$ ± 3.47	$15.75^{a,A}$ ± 4.32	$13.34^{a,b,B}$ ± 3.83	$15.86^{a,c,A}$ ± 4.53
0.5% whey isolate	$12.35^{a,A}$ ± 2.78	$12.55^{a,A}$ ± 3.70	$12.53^{b,A}$ ± 3.44	$12.49^{a,A}$ ± 2.45	$15.55^{a,B}$ ± 4.09
1.5% whey isolate	$14.39^{a,b,A,B}$ ± 3.86	$12.94^{a,A}$ ± 3.11	$16.25^{a,B}$ ± 4.83	$13.58^{a,b,A}$ ± 2.35	$12.58^{b,A}$ ± 4.29
0.5% soy isolate	$14.79^{b,A}$ ± 3.24	$15.88^{b,A,B}$ ± 3.65	$17.85^{a,B}$ ± 5.43	$13.99^{a,b,A}$ ± 4.12	$18.00^{c,B}$ ± 3.54
1.5% soy isolate	$14.20^{a,b,A,C}$ ± 3.30	$13.93^{a,b,A}$ ± 3.17	$17.09^{a,B}$ ± 3.06	$14.83^{b,A,C}$ ± 3.96	$16.01^{a,c,B,C}$ ± 4.23

a–c values in the same column with different superscripts are significantly different ($P < 0.05$)
A–C values in the same row with different superscripts are significantly different ($P < 0.05$)

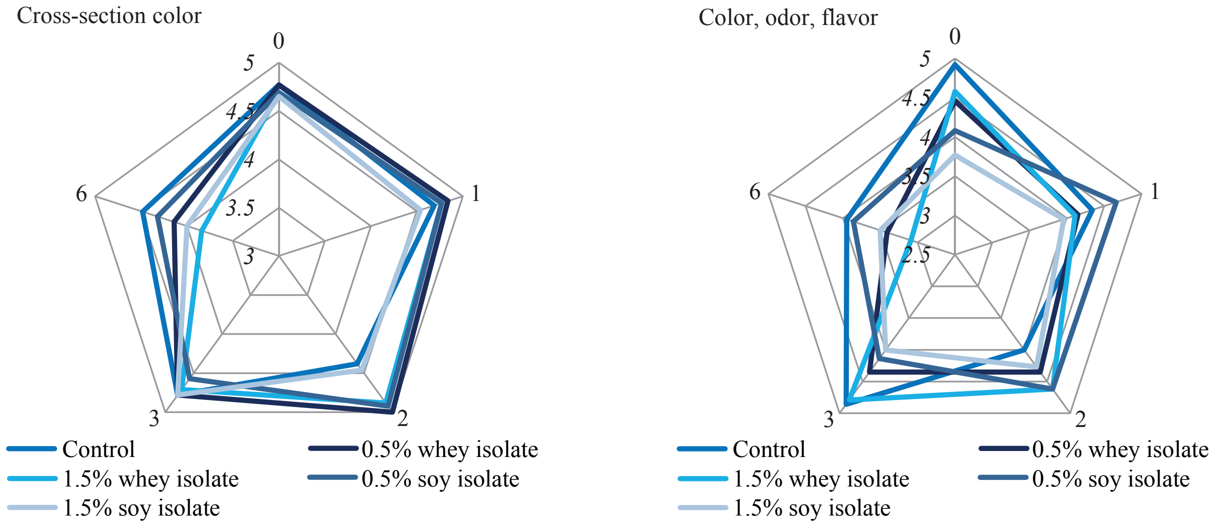

Figure 1 Sensory evaluation of cross-section color, odor, flavor, and taste of sausages with protein isolates

damaged; it was slightly wrinkled and highly graded for all the samples. Similar results were obtained by Vasilev *et al.* for a functional sausage with fatty tissue [39].

After production, the cross-section of the samples had a mosaic appearance, with slightly larger particles of fat tissue, which is characteristic of this product. Inside the sausage were no visible cracks, and the components were well connected. The appearance of the section was rated very highly in all the test samples during storage. The lowest values were recorded, as expected, after 6 months of storage, from 4.36 to 4.86. Most panelists pointed out the presence of a high content of fatty tissue at the intersection of the sausage, which was confirmed by an extremely high fat content in the samples. Bratulić *et al.* made the same conclusion, having examined sausages from the Istrian region [12].

The sensory evaluation of the cross-section color, as well as odor, flavor, and taste in the analyzed sausage samples during a six-month storage period are presented in Fig. 1. The cross-section color after production was adequate, with minimal deviations. The meat pieces were red and the particles of fat tissue were whitish. The grades ranged from 4.65 to 4.77, and later, between the 1st and the 3rd months, they varied from 4.38 to 5.00. At the end of the test period, after six months of storage, the ratings were lower, ranging from 3.84 to 4.48, but they were still acceptable. The color was noticeably lighter in the samples with soy protein, although the other samples were characterized as slightly brighter than expected (pieces of meat). After six months, a greater change in color was noticeable, especially at the edges, which was more expressed in the whey samples.

Abdolghafour and Zaki cited higher grades for the samples with added whey, which declined during storage [18, 35]. According to Akesowana, adding soybeans had a positive effect on the color, while Krasnowska *et al.* did not indicate a significant difference between the samples with soy and whey compared to the control sample [13, 34].

The most obvious changes in sensory characteristics were in odor, taste, and flavor during the storage period. We found that the use of additives hardly affected the characteristic pleasant smell of fermented products after ripening and the mild smell of smoke. The grades after production ranged from 3.77 to 4.92. Adding soy proteins during this period reduced the intensity of aroma and flavor, contributing to a bland taste. Many authors reported similar observations: adding up to 3% of soy protein masked the intensity of other flavors, reduced juiciness and salinity [40]. Serdaroglu concluded that whey caused the absence of meat flavor [17]. We found changes in sensory properties during storage. The samples with 1.5% of additives had less expressed characteristics, a mismatch of aroma and taste. The whey samples had a sour odor. Krasnowska *et al.* cited slightly lower grades for flavor and juiciness, and better grades for taste in the samples with soy and whey proteins [13, 30, 34].

The texture of the sausages after production was satisfactory, with minor deviations (Fig. 2). Observing a sausage cut, we found that the mass was compact and that the additives had a noticeable effect on chewiness. The ratings after production were from 4.00 to 4.92. During storage, there were changes in texture, with the grades ranging from 3.92 to 4.57. The whey samples became softer and less connected, while the soy samples were harder than expected. After six months, the grades were lower, ranging from 3.45 to 4.23. The products crumbled during the cutting and also demonstrated some toughness. The samples with a higher amount of added protein attained lower grades, while the 0.5% soy protein sample had the best texture. Many authors reported a positive effect of added soy and whey on the texture, and therefore on the grades, compared to the control sample [13, 18, 34].

The overall sensory quality of all the samples was quite high during the entire test period, with no major

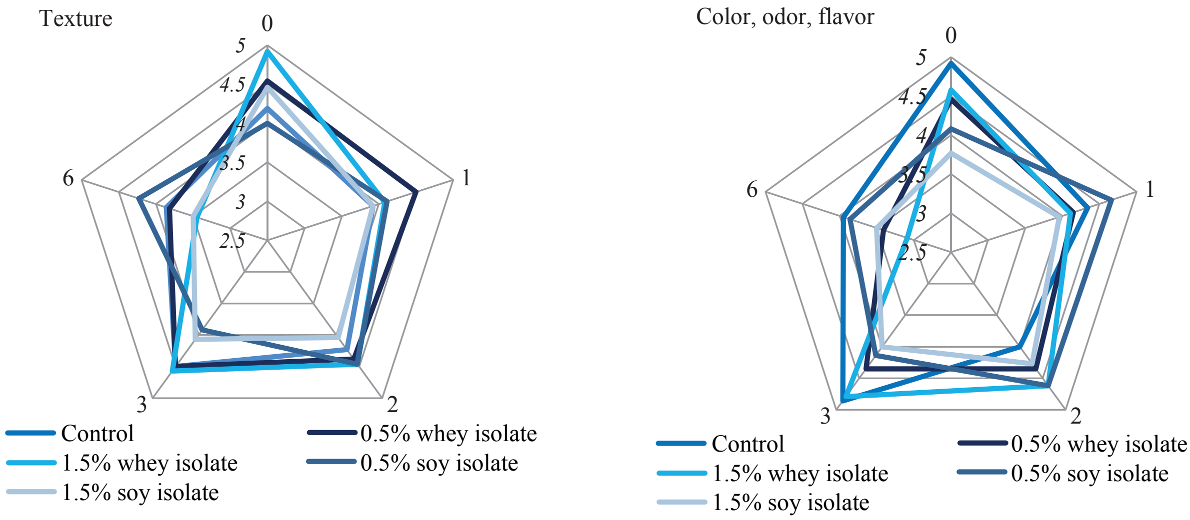

Figure 2 Texture and overall sensory quality of sausages with protein isolates

deviations from the maximum quality ($P > 0.05$). After production, the grades ranged from 4.37 to 4.79. The whey samples received high grades (4.71), almost as the control (4.77), while the soy samples rated slightly lower (4.37 and 4.44, respectively). During the storage period, there were no major changes, with the mean scores ranging from 4.35 to 4.82, and the samples with smaller amounts of additives were rated slightly better. After six months of storage, the marks were somewhat lower (3.98 to 4.32). In this period, the soy samples were given better grades, just as the samples with smaller amounts of additives We found that all the samples showed good ratings and acceptability during the entire test period.

Krasnowska *et al.* found that the products with whey and soy proteins had better sensory parameters [34]. Many authors agree that whey and soy protein supplements have a positive influence on sensory characteristics [18, 30, 40].

CONCLUSION

The results of our study showed that protein supplements possessed excellent functional properties in fermented products, including the emulsifying and binding properties. We found a significantly noticeable reduction of water activity, which is very important in the production of fermented sausages in terms of the ripening rate. Slower moisture losses during storage were observed in the samples with additives. Another effect was that on hardness: a 0.5% soy protein isolate resulted in a tougher product. We also found a minor effect on the color: the samples with the additives showed a slightly lower L^* value, while those with soy protein had higher yellowness (b^*). The use of the additives did not have a significant effect on the chemical composition and sensory properties of the product ($P > 0.05$) because of their low concentrations. The main characteristics of meat products were preserved despite the addition of non-meat proteins.

However, the effect of non-fat proteins on the quality of fermented sausages needs further investigation to determine the optimal concentration for obtaining high quality products.

CONTRIBUTION

Authors are equally related to the writing of the manuscript and are equally responsible for plagiarism.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests.

REFERENCES

1. Vasilev D, Aleksic B, Tarbuk A, Dimitrijevic M, Karabasil N, Cobanovic N, et al. Identification of lactic acid bacteria isolated from Serbian traditional fermented sausages Sremski and Lemeski kulen. Procedia Food Science. 2015;5:300–303. DOI: https://doi.org/10.1016/j.profoo.2015.09.071.
2. Yadav SK, Tanwar VK, Sharma JK, Yadav S. Effect of added soy protein on physico-chemical properties of chevon patties. Journal of Meat Science and Technology. 2013;1(1):35–39.
3. Akpan IP. Trends in sausage production. African Journal of Food Science and Technology. 2017;8(5):081–084. DOI: https://doi.org/10.14303/ajfst.2017.089.
4. Skaljac S, Jokanovic M, Tomovic V, Ivic M, Tasic T, Ikonic P, et al. Influence of smoking in traditional and industrial conditions on colour and content of polycyclic aromatic hydrocarbons in dry fermented sausage “Petrovska klobasa”. LWT – Food Science and Technology. 2018;87:158–162. DOI: https://doi.org/10.1016/j.lwt.2017.08.038.

5. Petrovic L, Tasic T, Ikonic P, Sojic B, Skaljac S, Danilovic B, et al. Quality standardization of traditional dry fermented sausages: Case of Petrovska klobasa. In: Nedović V, Raspor P, Lević J, Tumbas Šaponjac V, Barbosa-Cánovas GV, editor. Emerging and Traditional Technologies for Safe, Healthy and Quality Food. Cham: Springer; 2016. pp. 221–234 DOI: https://doi.org/10.1007/978-3-319-24040-4_12.

6. Holck A, Axelsson L, McLeod A, Rode TM, Heir E. Health and safety considerations of fermented sausages. Journal of Food Quality. 2017. DOI: https://doi.org/10.1155/2017/9753894.

7. Gujral HS, Kaur A, Singh N, Sodhi NS. Effect of liquid whole egg, fat and textured soy protein on the textural and cooking properties of raw and baked patties from goat meat. Journal of Food Engineering. 2002;53(4):377–385. DOI: https://doi.org/10.1016/S0260-8774(01)00180-7.

8. Dzinic N, Ivic M, Sojic B, Jokanovic M, Tomovic V, Okanovic D, et al. Some quality parameters of dry fermented sausages (*Cajna kobasica*). Procedia Food Science. 2015;5:77–80. DOI: https://doi.org/10.1016/j.profoo.2015.09.019.

9. Seong PN, Seo HW, Kang SM, Kim YS, Cho SH, Kim JH, et al. Beneficial Effects of Traditional Seasonings on Quality Characteristics of Fermented Sausages. Asian-Australasian Journal of Animal Sciences. 2016;29(8):1173–1180. DOI: https://doi.org/10.5713/ajas.15.0738.

10. Pagthinathan M, Nafees MSM. Physicochemical properties and sensory evaluation of fermented sausage using probiotic bifidobacterium. International Journal of Research Publication. 2018;12(1).

11. Škaljac S. Uticaj različitih tehnoloških parametara na formiranje boje tradicionalne fermentisane kobasice (*Petrovačka kobasica*) tokom standardizacije bezbednosti i kvaliteta [Utication of the different technological parameters on the formation of the buoy of traditional fermented grasshopperhead (kobasica Petrovic) flow of efficiency i quality standardisation]. Dr. eng. sci. diss. Novi Sad: University of Novi Sad; 2014. 355 p.

12. Bratulić M, Fleck ŽC, Mikuš T, Njari B, Kozačinski L. Proizvodnja i kakvoća domaćih istarskih kobasica [Production and quality of home – made Istrian dry sausages]. MESO: Prvi hrvatski časopis o mesu [MESO: The first Croatian meat journal]. 2011;13(4):262–264. (In Croatian).

13. Akesowan A. Effect of soy protein isolate on quality of light pork sausages containing konjac flour. African Journal of Biotechnology. 2008;7(24):4586–4590.

14. Grasso S, Smith G, Bowers S, Ajayi OM, Swainson M. Effect of texturised soy protein and yeast on the instrumental and sensory quality of hybrid beef meatballs. Journal of Food Science and Technology. 2019;56(6):3126–3135. DOI: https://doi.org/10.1007/s13197-018-3552-9.

15. Vasilev D, Glisic M, Jankovic V, Dimitrijevic M, Karabasil N, Suvajdzic B, et al. Perspectives in production of functional meat products. IOP Conference Series: Earth and Environmental Science. 2017;85. DOI: https://doi.org/10.1088/1755-1315/85/1/012033.

16. Suvajdžić B. Ispitivanje mikroflore i parametara kvaliteta sremskog kulena proizvedenog u industrijskim i tradicionalnim uslovima [Microbiota and quality parameters of Sremski kulen produced in industrial and traditional conditions]. Belgrade: University of Belgrade; 2018. 130 p. (In Croatian).

17. Serdaroğlu FM, Fadiloğlu EE. Chemical composition and quality characteristics of emulsion type turkey rolls formulated with dairy ingredients. Journal of Food Technology. 2004;2(2):109–113.

18. Abdolghafour B, Saghir A. Effect of whey protein concentrate on quality and shelf life of buffalo meat emulsion sausage. Scholars Journal of Agriculture and Veterinary Sciences. 2014;1(4):201–210.

19. Kovačević D, Mastanjević K, Ćosić K, Pleadin J. Količina nitrita i nitrata u mesnim proizvodima s hrvatskog tržišta [Quantity of nitrites and nitrates in meat products from the Croatian market]. MESO: Prvi hrvatski časopis o mesu [MESO: The first Croatian meat journal]. 2016;18(2):40–46. (In Croatian).

20. Karolyi D, Salajpal K, Luković Z, Kovačić D. Physicochemical, hygienic and organoleptic characterisation of Slavonian Kulen, a traditional pork sausage from eastern Croatia. Options Méditerranéennes: Série A. Séminaires Méditerranéens. 2012;(101):281–288.

21. Mastanjevic K, Kovacevic D, Frece J, Markov K, Pleadin J. The effect of Autochthonous starter culture, sugars and temperature on the fermentation of Slavonian Kulen. Food Technology and Biotechnology. 2017;55(1):67–76. DOI: https://doi.org/10.17113/ftb.55.01.17.4688.

22. Operta S, Leko N, Tahmaz J, Alkić M. Ispitivanje fizikalno-kemijskih i senzornih svojstava "Vlašićke" kobasice tijekom čuvanja [Investigation of physicochemical and sensory properties of "Vlašićka" sausage during storage]. MESO: Prvi hrvatski časopis o mesu [MESO: The first Croatian meat journal]. 2015;17(3):247–253. (In Croatian).

23. Menegas LZ, Pimentel TC, Garcia S, Prudencio SH. Dry-fermented chicken sausage produced with inulin and corn oil: Physicochemical, microbiological, and textural characteristics and acceptability during storage. Meat Science. 2013;93(3):501–506. DOI: https://doi.org/10.1016/j.meatsci.2012.11.003.

24. Hussein FH, Razavi SH, Emam-Djomeh Z. Physicochemical properties and sensory evaluation of reduced fat fermented functional beef sausage. Applied Food Biotechnology. 2017;4(2):93–102. DOI: https://doi.org/10.22037/afb.v4i2.15181.
25. Stajic S, Stanisic N, Levic S, Tomovic V, Lilic S, Vranic D, et al. Physico-chemical characteristics and sensory quality of dry fermented sausages with flaxseed oil preparations. Polish Journal of Food and Nutrition Sciences. 2018;68(4):367–375. DOI: https://doi.org/10.2478/pjfns-2018-0006.
26. Bolumar T, Toepfl S, Heinz V. Fat reduction and replacement in dry-cured fermented sausage by using high pressure processing meat as fat replacer and olive oil. Polish Journal of Food and Nutrition Sciences. 2015;65(3):175–182. DOI: https://doi.org/10.1515/pjfns-2015-0026.
27. Lee CW, Kim TK, Hwang KE, Kim HW, Kim YB, Kim CJ, et al. Combined effects of wheat sprout and isolated soy protein on quality properties of breakfast sausage. Korean Journal for Food Science of Animal Resources. 2017;37(1):52–61. DOI: https://doi.org/10.5851/kosfa.2017.37.1.52.
28. Priyadarshi P. Comparative study of the effects of three food proteins on the cooking quality of pork sausages. Dr. eng. sci. diss. 2013. 116 p.
29. Andres S, Zaritzky N, Califano A. The effect of whey protein concentrates and hydrocolloids on the texture and colour characteristics of chicken sausages. International Journal of Food Science and Technology. 2006;41(8):954–961. DOI: https://doi.org/10.1111/j.1365-2621.2005.01152.x.
30. Andic S, Zorba O, Tuncturk Y. Effect of whey powder, skim milk powder and their combination on yield and textural properties of meat patties. International Journal of Agriculture and Biology. 2010;12(6):871–876.
31. Barros DB, Sobrosa C, Pinheiro R, Fonseca S, Vaz-Velho M. Effect of addition of a starter cultures on physicochemical and sensory properties of “Alheira”, a smoked sausage-like product. Millenium. 2018;2(7):39–48. DOI: https://doi.org/10.29352/mill0207.03.00195.
32. Kim J-H, Pyun C-W, Hong G-E, Kim S-K, Yang C-Y, Lee C-H. Changes in physicochemical and microbiological properties of isoflavone-treated dry-cured sausage from sulfur-fed pork during storage. Journal of Animal Science and Technology. 2014;56. DOI: https://doi.org/10.1186/2055-0391-56-21.
33. Glisic M, Baltic M, Trbovic D, Jokanovic M, Parunovic N, Dimitrijevic M, et al. Inulin-based emulsion-filled gel as a fat replacer in prebiotic- and PUFA-enriched dry fermented sausages. International Journal of Food Science and Technology. 2019;54(3):787–797. DOI: https://doi.org/10.1111/ijfs.13996.
34. Krasnowska G, Rudownik E, Sobków B, Gęsikowska A. Quality of comminuted sausages made with functional proteins. Polish Journal of Food and Nutrition Sciences. 2005;14(1):85–89.
35. Zaki EF. The quality characteristics of camel sausage formulated with different levels of whey protein powder. International Journal of Environment, Agriculture and Biotechnology. 2017;2(5):2481–2486. DOI: https://doi.org/10.22161/ijeab/2.5.27.
36. Hughes E, Cofrades S, Troy DJ. Effects of fat level, oat fibre and carrageenan on frankfurters formulated with 5, 12 and 30% fat. Meat Science. 1997;45(3):273–281. DOI: https://doi.org/10.1016/s0309-1740(96)00109-x.
37. Barbut S. Effects of milk powder and its components on texture, yield, and color of a lean poultry meat model system. Poultry Science. 2010;89(6):1320–1324. DOI: https://doi.org/10.3382/ps.2009-00506.
38. Skaljac S, Jokanovic M, Tomovic V, Ivic M, Sojic B, Ikonic P, et al. Colour characteristics of vacuum packed fermented sausage during storage. IOP Conference Series: Earth and Environmental Science. 2019;333. DOI: https://doi.org/10.1088/1755-1315/333/1/012101.
39. Vasilev D, Vuković I, Tomović V, Jokanović M, Vasiljević N, Milanović-Stevanović M, et al. Važnije fizičke, fizičko-hemijske i senzorske osobine kvaliteta funkcionalnih fermentisanih kobasica [More important physical, physico-chemical and sensory qualities of functional fermented sausages]. Tehnologija mesa [Technology of meat]. 2009;50(5–6):342–350. (In Croatian).
40. Mapanda C, Hoffman LC, Mellett FD, Muller N. Effect of pork rind and soy protein on polony sensory attributes. Journal of Food Processing and Technology. 2015;6(2). DOI: https://doi.org/10.4172/2157-7110.1000417.

ORCID IDs
Ana Velemir https://orcid.org/0000-0003-2152-5183
Danica Savanović https://orcid.org/0000-0001-7672-111X