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Effect of the Addition of Brewers' Spent Grain (BSG) on the Physicochemical and Consumer Liking Attributes of Croatian Indigenous Cooked Sausage "Bijela Krvavica"

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Featured Application: The utilization of fresh (wet) brewers' grains (BSG) in the formulation of cooked sausages enables a more economical production process and utilization of BSG in human nutrition (e.g., β-glucan intake).

Abstract: This research aimed to investigate the effect of the addition of wet brewers' spent grains (BSG) on the microbiological safety, and physicochemical and sensory attributes of "bijela krvavica" during seven-day storage at 4 °C. The addition of BSG to the samples of "bijela krvavica" statistically significantly ($p < 0.05$) affected the mass share of proteins and fat and increased the mass share of water. Increasing the mass share of BSG in "bijela krvavica" did not have a statistically significant ($p < 0.05$) effect on the mass share of collagen, or on the pH value and a_w. Instrumentally determined lightness (L*), redness (a*), and yellowness (b*) showed a decrease with the increase in mass share of wet BSG. Texture profile analysis (TPA) parameters hardness and chewiness also showed a decrease with the increase in BSG share (w = 0–9%) with statistical significance ($p < 0.05$). The overall liking of "bijela krvavica" samples showed the highest scores for the control sample, while 3% added BSG was rated as second best with no significant difference ($p < 0.05$). According to the consumer-generated sensory attributes (CATA) scoring, samples with 6 and 9% addition of BSG were described with attributes considered undesirable for meat products, such as "wheaty", "pale color", and "poor mouthfeel". Also, the LogCFUg^{-1} of enterobacteria, sulfite-reducing clostridia, *Staphylococcus aureus*, *Salmonella* spp., and *Listeria monocytogenes* were not affected. The data obtained in this research indicate the possibility of producing safe "bijela krvavica" sausages with wet brewers' spent grains without major changes in the physicochemical and sensory properties.

Keywords: "bijela krvavica"; BSG; microflora; sensory liking; instrumental color and texture

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1. Introduction

Sausages are commonly distinguished by the temperature treatment during processing and thus can be designated as dry or cooked [1]. Cooked sausages represent a large group of heat-treated meat products with different organoleptic properties. Cooked sausages are mainly made from beef and pork and other ingredients such as adipose tissue, pork skin, organs, water, spices, and extenders such as starches and flours with additives (salt, phosphates, citrates, ascorbates, nitrite, glutamate, lactates, and glucono-delta-lactone) [2,3]. Due to different ingredients, heat treatments, and storage times, cooked sausages are prone to chemical, microbiological, and sensory deterioration [4,5].

Traditional Croatian cooked sausages are mainly produced from low-quality pig meat, pig offal (liver, heart, lungs, etc.), skin, blood, and other ingredients. "Bijela krvavica" is a

traditional Croatian cooked sausage produced from cooked pig head tissue, cooked pig heart, cooked pig tongue, and cooked pig skins, salt, and spices (red paprika powder, hot paprika powder, garlic, and black pepper) [6].

Trends in the food industry demand the incorporation of plant-based materials into foods, even in meat products. Even though meat is an excellent protein source, and is highly nutritious, it is also poor in fiber content. Thus, numerous investigations have been conducted in order to incorporate various plant waste materials into meat products, many of which are described in Muntean et al. [7] and Younis et al. [8]. Younis and Ahmad [9] reutilized apple pomace as a functional ingredient in buffalo sausages to serve as a source of fiber. In a review by Younis et al. [8], the authors described the addition of soluble dietary fibers to meat products and how it affects the texture of such products. Similarly, Fogarasi et al. [10] added different shares of spent grain flour to smoked sausages. The result was nutritive added value, while the sensory analysis showed liking for samples with up to 5% BSG powder.

Brewers' spent grain (BSG) along with spent hops, and spent yeasts are the most important byproducts of beer production [11]. BSG is the most important beer industry byproduct with an amount of about 85% [11]. The world beer industry produces about 39 million tons of BSG per year, of which the EU produces 3.4 million tons. It is produced all year by large and small breweries. BSG mainly consists of husks (pericarp seed layers) from the original barley grain with a small amount of starch (depending on the mashing efficiency) and walls of empty aleurone cells. BSG is a lignocellulosic material, which mainly consists of fiber (hemicellulose and cellulose), protein, and lignin [12–14].

During the production of 1 hL of beer, about 21–22 kg of BSG, containing 70 to 80% water, is obtained. BSG in this form is mostly used as cattle feed. Due to its moisture and polysaccharide content, it is susceptible to microbiological spoilage. The most used methods for BSG preservation are oven-drying, freeze-drying, freezing, and the use of superheated steam [11].

These methods for BSG preservation are energy-consuming and result in high costs [7]. Fresh BSG sells for about EUR 35 per ton, while dry BSG achieves a price of about EUR 400 per ton [15]. The use of chemicals (lactic, acetic, formic, and benzoic acids) to prolong the shelf life of BSG is also an option but they are not positively perceived by consumers [11].

Since drying BSG is time- and energy-consuming, the utilization of wet BSG would be more economical. Plus, this would add value to the nutritive score of meat sausages and keep up with the trend of replacing meat raw materials with environmentally friendly plant waste materials. Thus, the aim of this research was to investigate the possibility of the utilization of wet BSG for the production of "bijela krvavica", a traditional Croatian cooked sausage.

2. Materials and Methods

2.1. Sausage Preparation

The wet BSG was acquired from a local craft brewery (Beckers Craft Brewery, Osijek, Croatia) immediately after lautering and stored for 1 day at 4 °C before the analysis and sausage preparation.

The process of making "bijela krvavica" sausages started with cooking the pigs' heads, tongues, and hearts (Figure 1) purchased at MM Mesna Industrija, Krašić, Croatia. During cooking, bay leaf (several leaves) and black pepper grains (a dozen grains) were added, and 1 h before the end of cooking, pork skin was added. When the entire mixture cooled down, the whole mass was ground (8 mm plate holes), and then additives and spices (purchased at Derma d.o.o., Varaždin, Croatia) were added to the mixture. Table 1 shows the recipe with the basic ingredients, spices, and additives used in making "bijela krvavica".

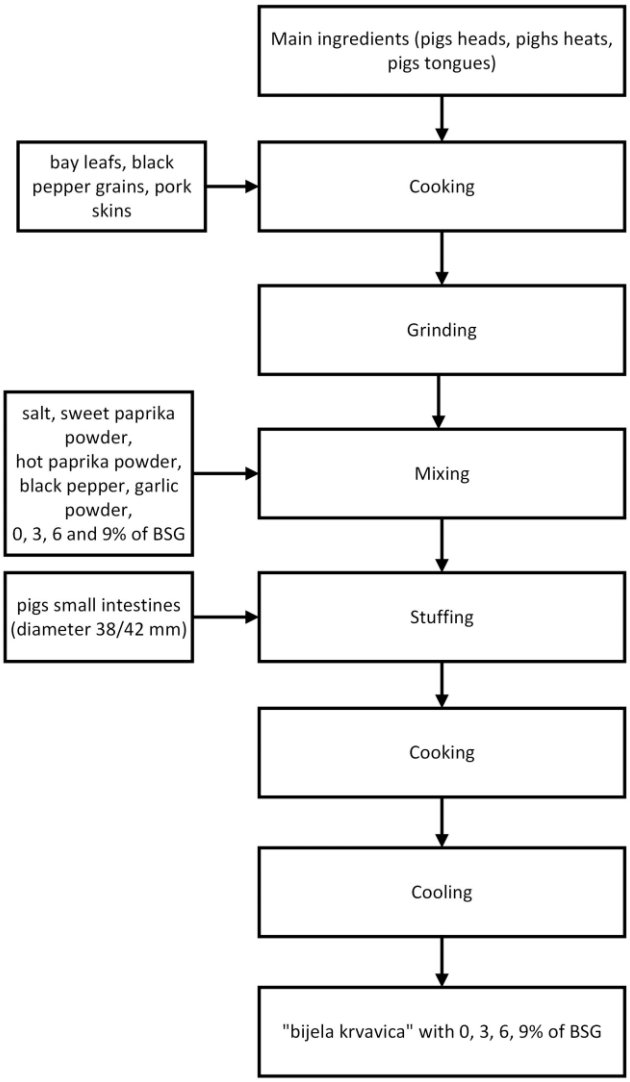

Figure 1. Flowchart of "bijela krvavica" sample production.

Table 1. Recipe with the basic ingredients, spices, and additives used in making "bijela krvavica".

	Mass Share (%)	Source
Meat of pig's heads	30.0	Local market
Pig hearts	25.0	
Pig tongues	35.0	
Pig skins	15.8	
Salt	2.0	Local market
Sweet paprika powder	0.6	Local producer
Hot paprika powder	0.6	Local producer
Black pepper	0.5	Local market
Garlic powder	0.5	Local market

The prepared stuffing was then separated into four parts, to which 0, 3, 6, and 9% BSG were added. After that, it was stuffed into pigs' small intestines (diameter 38/42 mm). After filling, sausages were cooked at a temperature of 80 °C for 10 min and then quickly cooled with ice water to a temperature of 4 °C. With the cooling process, the technological process of the production of cooked sausages is completed. After that, the samples were packed in polyethylene bags and stored for 1, 3, 5, and 7 days at 4 °C.

2.2. Analytical Methods

2.2.1. Physical–Chemical Analysis

All samples were homogenized using a knife mill Gridomix GM 200 (Retsh, Haan, Germany). The wet BSG was analyzed for water, protein, and ash content according to the American Association of Cereal Chemists (AACC) methods [16]. Also, the total dietary fiber was determined according to the AACC standard method (Method No. 32-07) [17].

The pH of the sausage samples was measured with a ProfiLine pH 3210 pH meter (WTW Wissenschaftlich-Technische Werkstätten GmbH, Weilheim in Oberbayern, Germany), according to the ISO 2917:1999. a_w was measured using a Rotronic Hygrolab 3 (Rotronic AG, Bassersdorf, Switzerland).

A Hunter-Lab Mini ScanXE (A60-1010-615 Model Colorimeter, Hunter-Lab, Reston, VA, USA) was used for instrumental color (L* lightness; greenness (−a*) or redness (a*); and blueness (−b*) or yellowness (b*)) measurements.

Determination of moisture content was performed according to ISO 1442:1997 [18] (Memmert UFE 400, Memmert, GmbH Germany, Schwabach, Germany). The Kjeldal method (ISO 937:2023) [19] was used to determine total protein content. Total fat content was determined according to ISO 1443:1973 [20] (Soxlet method). The determination of collagen content was performed through the analysis of hydroxyproline according to ISO 3496:1994 [21], using a spectrophotometer (Shimadzu, UV-1800., Tokyo, Japan).

Texture profile analysis (TPA) was performed using a TA.XT2i SMS Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd., Surrey, England) equipped with a P/75 aluminum cylindrical probe. This involved cutting the samples into 1.5 cm thick slices and their double compression so as to downsize them to 40% of their original thickness. The following parameters were quantified [22]: hardness (g), springiness (ratio), cohesiveness (ratio), and chewiness (g).

2.2.2. Microbiological Analysis

Microbiological analyses were performed on three samples from each group of sausages and wet BSG in duplicate. The following microbial analyses were performed: total viable count (TVC) (ISO 4833-1:2013) [23], *Enterobacteriaceae* (ISO 21528-2:2017) [24], sulfite-reducing clostridia count (ISO 15213-1:2023) [25], *Salmonella* spp. (ISO 6579-1:2017) [26], and *Listeria monocytogenes* (ISO 11290-1:2017) [27]. The results were expressed as a LogCFUg^{-1}.

2.2.3. Sensory Analysis

Sensory attributes for the CATA (check-all-that-apply) analysis [28] were defined as related to the texture (dry, soft, greasy, hard, and poor mouthfeel), flavor (meaty, wheaty, and off-flavor), and appearance (pale color, dark, and fatty). They were generated and adapted from Neville et al. [29] using the desk research method [30]. Consumers (n = 71; M = 15, F = 56) aged 19 to 60 years were recruited from the faculty (students and staff). Only meat consumers were selected. Sensory analysis of sausages prepared with the addition of BSG (plus control) was conducted after 7 days of storage at 4 °C. Samples were served sliced (10 mm slices) and each panelist received 3 slices (every slice was approx. 15 g). Samples were served uniformly on white paper plates marked with randomly selected three-digit codes and served after they had been exposed to room temperature (22 °C) for 15 min. The order of presentation of the samples and tests was determined by a randomized balanced design.

For each sample, the consumers were first asked to score their overall liking using a vertical 9-point hedonic scale anchored at “dislike extremely” (1) and “like extremely” (9) (overall liking) with (5) being designated as “neither liked nor disliked”. Next, they completed the CATA questionnaire with the 11 terms related to the sensory attributes of the samples. The consumers were asked to try the sample and then check all the terms they considered appropriate to describe each sample. All testing was performed in a separate sensory testing booth, under appropriate lighting, air, temperature, and humidity conditions.

2.3. Statistical Analysis

The results of the physicochemical evaluation of "bijela krvavica" samples are presented as mean values (three determinations for pH, a_w, moisture, fat, protein, and collagen content; five determinations for TPA parameters and instrumental color parameters). Analysis of variance of physicochemical properties and overall liking (one-way ANOVA) and then Fischer's LSD least significant difference test were performed using Statistica 13.0 (TIBCO Software Inc., Palo Alto, CA, USA), and statistically significant differences were expressed at the probability level of 95% ($p < 0.05$).

The frequency of use of each sensory attribute in the CATA questionnaire was determined as a sum of consumers that used that term to describe a sample. The collected data were subjected to Cochran's Q test to define the significant differences between samples for each of the terms included in the CATA questionnaire. Cochran's Q test was performed using XLStat–Pro (Addinsoft, Paris, France).

3. Results and Discussion

The wet BSG contained 76.30% water, 21.21% protein (DM), and 4.40% ash (DM). The samples of BSG showed a high total dietary fiber content of 64.9% (DM). This is in accordance with the previous studies of BSG composition [31,32]. The BSG has a high content of protein and total dietary fiber which makes it suitable as a cooked sausage ingredient.

The physicochemical properties of "bijela krvavica" produced during storage for seven days at a temperature of 4 °C are shown in Table 2. The largest mass share of proteins and fat (18.77% and 27.97%) is shown for samples without the addition of BSG stored for 7 days at 4 °C. On the other hand, the largest mass share of water is shown for the sample with the addition of 9% of wet BSG after 1 day of storage. The obtained results show that with the increase in the mass share of BSG, the mass share of proteins and fat in the samples decreased and the mass share of water increased statistically significantly ($p < 0.05$) for all storage times at a temperature of 4 °C. This is apparently the result of the addition of wet BSG that contained 76.30% water.

Table 2. Chemical composition, pH, and a_w of "bijela krvavica" with the addition of BSG during cold storage (4 °C).

% BSG	% Protein	% Fat	% Water	% Collagen	pH	a_w
			Day 1			
0	18.10 ^{a}	27.12 ^{a}	48.88 ^{c}	3.78 ^{a}	6.52 ^{a}	0.948 ^{b}
3	17.24 ^{b}	25.78 ^{b}	51.47 ^{b}	3.53 ^{a}	6.52 ^{a}	0.951 ^{ab}
6	17.05 ^{bc}	24.14 ^{c}	52.90 ^{a}	3.71 ^{a}	6.56 ^{a}	0.956 ^{a}
9	16.71 ^{d}	22.50 ^{d}	53.30 ^{a}	3.86 ^{a}	6.47 ^{a}	0.956 ^{a}
			Day 3			
0	18.36 ^{a}	27.24 ^{a}	48.67 ^{a}	3.15 ^{a}	6.54 ^{a}	0.938 ^{b}
3	17.44 ^{b}	25.85 ^{b}	50.95 ^{c}	4.54 ^{a}	6.53 ^{a}	0.938 ^{b}
6	17.19 ^{c}	24.39 ^{c}	52.17 ^{b}	2.80 ^{a}	6.51 ^{a}	0.942 ^{a}
9	16.99 ^{d}	22.70 ^{d}	52.73 ^{a}	4.43 ^{a}	6.03 ^{a}	0.943 ^{a}
			Day 5			
0	18.67 ^{a}	27.58 ^{a}	48.25 ^{d}	5.15 ^{a}	6.59 ^{a}	0.934 ^{b}
3	17.57 ^{b}	26.05 ^{b}	49.96 ^{c}	4.35 ^{ab}	6.55 ^{b}	0.934 ^{b}
6	17.37 ^{c}	24.85 ^{c}	51.75 ^{b}	4.38 ^{ab}	6.54 ^{b}	0.936 ^{b}
9	17.11 ^{d}	24.40 ^{d}	52.45 ^{a}	3.22 ^{b}	6.57 ^{ab}	0.944 ^{a}
			Day 7			
0	18.77 ^{a}	27.97 ^{a}	46.37 ^{d}	5.17 ^{a}	6.56 ^{a}	0.932 ^{b}
3	17.73 ^{b}	26.22 ^{b}	49.27 ^{c}	4.97 ^{ab}	6.54 ^{a}	0.935 ^{b}
6	17.58 ^{b}	26.05 ^{c}	50.27 ^{b}	3.60 ^{ab}	6.55 ^{a}	0.943 ^{a}
9	17.34 ^{c}	25.06 ^{d}	51.65 ^{a}	3.32 ^{b}	6.53 ^{a}	0.943 ^{a}

Values are means obtained with three measurements. Values displayed in the same column and tagged with different letters ($^{a–d}$) are significantly different ($p < 0.05$).

Nagy et al. [33] reported a decrease in moisture and an increase in protein, fat, and ash content when dry BSG was added to the smoked pork sausages, which was in agreement with the results for the "bijela krvavica" samples. Also, Almeida et al. [34] reported an increase in moisture content in emulsified cooked sausages with 25, 50, 75, or 100% of their pork back fat content replaced by amorphous cellulose gel. Other studies showed that the addition of BSG [33], insect flour [35], or mealworm flour [36] significantly increased protein content in emulsion sausages and frankfurters, which can be also related to the chemical composition of these flours. The pH values of the sausages did not vary significantly ($p < 0.05$), for all treatments and storage times. The values of water activity, similar to pH, did not show significant dependency on BSG addition for all storage times. The highest mass share of collagen (5.17%) was recorded in the sample without the addition of beer waste after 7 days of storage at 4 °C. In general, the collagen content showed a decrease with the increase in BSG, but without statistical significance ($p > 0.05$).

The instrumentally determined color parameters of "bijela krvavica" samples with different mass shares of BSG during seven-day storage at 4 °C are shown in Table 3.

Table 3. Instrumental color and TPA parameters of "bijela krvavica" with the addition of BSG during cold storage (4 °C).

% BSG	L*	a*	b*	Hardness (g)	Springiness	Cohesiveness	Chewiness (g)
			1 day				
0	47.10 ^{a}	16.07 ^{a}	28.48 ^{a}	15,141.47 ^{a}	0.84 ^{a}	0.58 ^{a}	7388.69 ^{a}
3	46.13 ^{a}	16.08 ^{a}	28.78 ^{a}	11,249.86 ^{b}	0.71 ^{a}	0.58 ^{a}	4693.57 ^{b}
6	46.62 ^{a}	16.64 ^{ab}	27.86 ^{a}	9750.94 ^{c}	0.68 ^{a}	0.58 ^{a}	3793.47 ^{c}
9	47.00 ^{a}	14.45 ^{b}	26.46 ^{b}	9045.02 ^{d}	0.68 ^{a}	0.58 ^{a}	3661.43 ^{d}
			3 days				
0	44.62 ^{bc}	15.15 ^{a}	28.15 ^{a}	14,672.70 ^{a}	0.79 ^{a}	0.50 ^{a}	5799.50 ^{a}
3	45.81 ^{ab}	14.44 ^{b}	27.39 ^{a}	12,893.50 ^{b}	0.80 ^{a}	0.53 ^{a}	5465.18 ^{b}
6	46.38 ^{a}	12.50 ^{b}	25.37 ^{b}	10,339.24 ^{c}	0.78 ^{a}	0.52 ^{a}	4220.10 ^{c}
9	46.69 ^{a}	11.85 ^{c}	24.86 ^{b}	7182.67 ^{d}	0.74 ^{a}	0.38 ^{b}	2024.04 ^{c}
			5 days				
0	43.80 ^{b}	14.82 ^{a}	28.69 ^{a}	19,483.88 ^{a}	0.78 ^{a}	0.64 ^{a}	9470.50 ^{a}
3	44.93 ^{ab}	13.90 ^{b}	28.37 ^{a}	18,239.55 ^{ab}	0.83 ^{a}	0.54 ^{ab}	8147.72 ^{ab}
6	46.29 ^{a}	13.80 ^{b}	27.72 ^{ab}	14,868.94 ^{b}	0.80 ^{a}	0.52 ^{b}	6137.56 ^{b}
9	46.05 ^{a}	12.81 ^{c}	26.54 ^{b}	10,249.67 ^{c}	0.74 ^{a}	0.48 ^{b}	3742.50 ^{c}
			7 days				
0	45.38 ^{ab}	14.51 ^{a}	27.41 ^{a}	12,897.88 ^{a}	0.81 ^{a}	0.50 ^{a}	5259.02 ^{a}
3	45.95 ^{a}	14.14 ^{a}	27.03 ^{ab}	12,128.35 ^{ab}	0.80 ^{ab}	0.47 ^{ab}	4674.24 ^{a}
6	44.42 ^{ab}	14.07 ^{a}	25.99 ^{ab}	10,388.75 ^{b}	0.80 ^{ab}	0.47 ^{ab}	3959.72 ^{a}
9	43.82 ^{b}	12.73 ^{b}	25.21 ^{b}	7469.37 ^{c}	0.76 ^{b}	0.40 ^{b}	2290.87 ^{b}

Values are means obtained with three measurements. Values displayed in the same column and tagged with different letters ($^{a–d}$) are significantly different ($p < 0.05$).

The highest L* value (47.10) was obtained for cooked sausage without the addition of BSG after one day of storage at 4 °C, while the lowest L* value (42.98) was obtained for the "bijela krvavica" with the addition of 6% BSG stored for three days at 4 °C. In general, the addition of BSG did not statistically significantly ($p < 0.05$) affect the L* parameter for all storage times at 4 °C. A decrease in L* with the addition of BSG is in agreement with the study by Choi et al. [36], who reported a decrease in L* value with the addition of mealworm flour.

The highest a* value (16.64) was noted in the one-day-old cooked sausage with the addition of 6% BSG (at 4 °C), while the lowest a* value (11.85) was noted in the three-day-old cooked sausage with the addition of 6% BSG. The highest b* value (28.78) was determined in the one-day-old cooked sausage with the addition of 3% BSG, while the lowest b* value (24.86) was reported in the cooked sausage with the addition of 3% BSG stored for three days at 4 °C. The addition of BSG to "bijela krvavica" samples mainly led

to a decrease in their a* value and b* value, but without statistical significance ($p > 0.05$). Another study [36] showed decreases in L *, a*, and b* values with the addition of broccoli to sausages.

On the other hand, in the studies [33,36], the addition of dry BSG to sausages increased a* values. This can be related to the different formulations of these sausages (addition of BSG and insect flour).

The parameters of the textural profile analysis (TPA) of "bijela krvavica" with the addition of BSG during seven-day storage at 4 °C are shown in Table 3.

The highest hardness of 19,483.88 g was recorded for the sausage without the addition of BSG (control sample) stored for 5 days at 4 °C, while the lowest hardness (7182.67 g) was determined in the sausage with 9% BSG after 3 days of storage at 4 °C. In general, the addition of BSG showed a statistically significant ($p < 0.05$) reduction in hardness. The addition of BSG did not statistically significantly ($p > 0.05$) affect the springiness and cohesiveness for all periods of storage at 4 °C. This agrees with studies that investigated the addition of dry BSG and a combination of baby corn, broccoli, carrot, and dry BSG to the sausages [36,37]. These foodstuffs are also plant raw materials and have a similar share of fiber and moisture content. The dry BSG is similar in protein and fiber content. This is why they correlate well with our research. The chewiness parameter showed the same dependence on the addition of BSG and storage time at 4 °C as the hardness parameter.

The number of formed colonies LogCFUg^{-1} of BSG for enterobacteria, sulfite-reducing clostridia, and *Staphylococcus aureus* was <10, for aerobic mesophilic bacteria, 2.1×10^4, while *Salmonella* spp. and *Listeria monocytogenes* were not found in 25 g of the sample.

The number of colonies (LogCFUg^{-1}) of enterobacteria for all samples of "bijela krvavica" with the addition of wet BSG for all storage times at 4 °C was < 10 (Table 4). Similarly, all storage times showed that the number of colonies (LogCFUg^{-1}) of sulfite-reducing clostridia for all sausage samples with the addition of BSG (w = 0–9%) was < 10. This was also the case for the LogCFUg^{-1} of *Staphylococcus aureus* for all samples of cooked sausages with the addition of BSG (w = 0–9%), where the number was < 10. The number of colonies (LogCFUg^{-1}) of *Salmonella* spp. for all types of tested sausage samples and all storage intervals was 0; that is, *Salmonella* spp. was not found in 25 g of the sample. In a study of the addition of BSG to smoked sausages, Nagy et al. [33] also did not determine the presence of *Salmonella* spp. during storage. The number of colonies (LogCFUg^{-1}) of *Listeria monocytogenes* for all of the tested sausage samples (w = 0–9%) was 0; that is, *Listeria monocytogenes* was not found in 25 g of the sample.

The number of colonies (LogCFUg^{-1}) of aerobic mesophilic bacteria (TPC) was the highest in "bijela krvavica" with the addition of 9% BSG after seven-day storage at 4 °C (4.9×10^4). The control sample had the lowest number of aerobic mesophilic bacteria, for all storage times. On the other hand, Ahmad et al. [38] reported that chicken sausages without the addition of capsicum, carrot, spinach, purple cabbage, and oyster mushroom had the highest total plate count. This is probably a result of higher phenolic compound content in these sausages [39]. The addition of wet BSG (w = 0–9%) resulted in an increase in the number of colonies (LogCFUg^{-1}) of TPC for all storage intervals at 4 °C, which is most likely a consequence of the presence (LogCFUg^{-1}) of aerobic mesophilic bacteria in the wet BSG.

The sensory evaluation involved a vertical 9-point hedonic scale followed by CATA analysis. The results are presented in Tables 5 and 6. Overall liking of "bijela krvavica" with the addition of BSG after seven-day storage at 4 °C is presented in Table 5. Samples with the addition of 6 and 9% BSG showed significantly ($p < 0.05$) lower scores in overall liking than the control sample, while the sample with 3% addition of BSG was rated somewhat higher than samples with 6 and 9% BSG. According to Fisher's test, consumers did not report a noticeable difference between the control sample and the samples with the addition of 3% BSG. The lowest score (6.7) was obtained for the sample with the addition of 9% BSG. Similarly, the results published by Neville et al. [29]. reported lower scores for overall liking for meat-free sausages, while sausages made from meat scored higher overall liking

results. Research conducted by Jorge et al. [40] resulted in an overall liking of mortadella samples, where the best score was given to a sample subjected to smoking, while other samples scored lower. However, all samples were rated above 5 points, which is a kind of neutral point, "neither liked nor disliked". Similarly, this was the case in this research, where all scores given by the consumers were on the positive side of the liking scale, for all samples.

Table 4. Microbiological analyses of "bijela krvavica" with the addition of BSG during cold storage (4 °C).

% BSG	TPC (CFU g^{-1})	E (CFU g^{-1})	SRC (CFU g^{-1})	SA (CFU g^{-1})	S (CFU g^{-1})	LM (CFU g^{-1})
			1 day			
0	7.5×10^2	<10	<10	<10	-	-
3	9.6×10^3	<10	<10	<10	-	-
6	1.8×10^4	<10	<10	<10	-	-
9	2.3×10^4	<10	<10	<10	-	-
			3 days			
0	1.7×10^3	<10	<10	<10	-	-
3	2.9×10^4	<10	<10	<10	-	-
6	3.5×10^4	<10	<10	<10	-	-
9	4.1×10^4	<10	<10	<10	-	-
			5 days			
0	5.0×10^3	<10	<10	<10	-	-
3	1.3×10^4	<10	<10	<10	-	-
6	2.5×10^4	<10	<10	<10	-	-
9	3.7×10^4	<10	<10	<10	-	-
			7 days			
0	2.6×10^4	<10	<10	<10	-	-
3	3.9×10^4	<10	<10	<10	-	-
6	4×10^4	<10	<10	<10	-	-
9	4.9×10^4	<10	<10	<10	-	-

TPC—total plate count; E—*Enterobacteriaceae*; SRC—sulfite-reducing clostridia; SA—*Staphylococcus aureus*; S—*Salmonella* spp.; LM—*Listeria monocytogenes*; - —not found.

Table 5. Overall liking of "bijela krvavica" with the addition of BSG.

% BSG	Overall Liking Mean ± SD
0	$8.7^{a} \pm 1.5$
3	$7.7^{ab} \pm 2.3$
6	$7.3^{b} \pm 1.4$
9	$6.7^{b} \pm 1.7$

Overall liking with different superscripts (^{a,b}) is significantly different ($p < 0.05$).

Attribute frequencies for "bijela krvavica" samples with the addition of BSG are shown in Table 6. Samples showed large variations ($p < 0.05$) in 9 out of 10 attributes. The only sensory attribute that did not show any significant variation ($p > 0.05$) was related to appearance, designated as dark. The control sample was perceived as "greasy", "hard", "meaty", and "fatty". On the other hand, the sample with the addition of 9% BSG was perceived as "dry", "soft", "poor mouthfeel", "wheaty", and "pale". The "pale" attribute was related to the addition of BSG, while "dark" was associated with the control sample, which was made without the addition of BSG. It seems that the aforementioned attribute was not affected by the addition of BSG to the stuffing. The attributes that can be detected visually, such as "dark" and "pale color", are the ones that determine the perception of the product and influence the purchase behavior of consumers [41,42]. The highest score for "pale" was attributed to the sample with 9% BSG, which is in accordance with the previously conducted L*a*b* analysis (Table 3). Simultaneously, the control sample was rated with

the highest score for "dark", which is also confirmed by the L*a*b* analysis shown in Table 3. According to these results, it seems that "pale" or lighter (L*) color is not positively correlated with the liking of this particular product. Even though the control sample showed higher scores for some of the attributes, such as "greasy", "hard", "meaty", and "fatty", the results showed that the control sample was liked by the consumers. Similarly, the sample with the addition of 3% BSG was generally well liked by the consumers. Samples with higher additions of BSG (6 and 9%) were somewhat less accepted/liked by the consumers, as can be seen from Table 6. It seems that the attributes "dry", "soft", "poor mouthfeel", "wheaty", and "pale", associated with 9% BSG addition, were not positively correlated with the overall liking.

Table 6. Contingency table for CATA evaluation of significant differences between samples described by Cochran's Q test.

Attribute	% BSG			
	0	**3**	**6**	**9**
Dry	5	12	19 *	25 *
Soft	6	15	20 *	32 *
Greasy	34	21 *	15 *	8 *
Hard	38	22 *	12 *	7 *
Poor mouthfeel	7	12	28 *	35 *
Meaty	41	22 *	15 *	10 *
Wheaty	6	14	25 *	34 *
Off-flavor	7	13	17 *	23 *
Pale color	5	17 *	31 *	38 *
Dark	21	20	18	15
Fatty	41	30 *	24 *	17 *

* Significant variation ($p < 0.05$) in relation to the control sample.

4. Conclusions

This study showed that the addition of wet BSG decreased the protein and fat contents and increased the moisture content of "bijela krvavica". Although the addition of wet BSG caused a deterioration of some instrumentally measured color (L*, a*, and b*) and texture parameters (hardness and chewiness), and microbiological analysis results (TPC), the final product was a safe cooked sausage. Consumer testing showed that samples with the addition of 3% BSG are liked by consumers. The absence of a significant difference in liking between the control sample and the 3% BSG added product indicated that plant-based additions to such meat products could be liked by consumers and have potential as a novel product. This represents only a preliminary study, to assess the shelf life and optimize the mass share of added wet BSG to "bijela krvavica", and further investigations are necessary.

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