Article

Comparison of the Effect of Enhancing Dry Fermented Sausages with *Salvia hispanica* and *Nigella sativa* Seed on Selected Physicochemical Properties Related to Food Safety during Processing

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Abstract: The aim of the study is to compare the effects of *Salvia hispanica* (Chia) seed and *Nigella sativa* (Black Cumin) seed in traditionally produced dry fermented sausages with reduced nitrites on the changes in physicochemical parameters (water content, pH, and water activity), thioarbituric acid reactive substances (TBARS), color parameters, the content of nitrosopigments, and microbial counts throughout the production process. At the end of the processing, the content of biogenic amines was also determined. Five samples were analyzed during the 30-day production process: the control sample, samples with 1% and 2% additions of chia seed, and samples with 1% and 2% additions of black cumin seed. It was indicated that the addition of chia or black cumin did not exert any effect on water content or water activity changes in fermented sausages. At the end of production, the samples were characterized by low water activity, in the range of 0.798–0.813. The sausages with chia seeds were characterized by the lowest pH due to the highest proliferation of lactic acid bacteria. TBARS values did not alter in both control and black cumin sausages throughout the experiment. Microbiological analysis showed that the addition of chia or black cumin seed enhances the proliferation of the lactic acid bacteria and caused a reduction in the number of *Enterobacteriaceae* in comparison to the control.

Keywords: chia seeds; black cumin seeds; fermented sausage; biogenic amines; nitrosylmyoglobin; lactic acid bacteria

1. Introduction

Meat consumption has been increased considerably during the last few years because the world's population is continuously growing, as well as due to rapid industrialization. The global meat production predicted in the coming years will rise drastically, and therefore the meat industry should meet the demand for these needs. This sector is considered one of the most important worldwide, and its production in 2019 was around 337 million tonnes. Europe represents about one-fifth of the world's production, providing 64 million tonnes, of which 5 million tonnes correspond to Poland [1]. Meat constitutes a significant part of the human diet due to its nutritional value. Although the meat consumption has a negative impact on the environment and is classified as a probable human carcinogen [2], it is an exceptional source of valuable nutrients, including highly bioavailable essential amino acids, vitamins, and minerals [3]. However, its consumption is subjected to consumer

concerns, lifestyles, and preferences, as well as cultural and geographical factors. In this evolution, the demand to extend meat shelf-life is increasing since it is related to safety and quality assurance. The fermentation process is one of the techniques traditionally employed to extend the shelf life of meat, and it is catalyzed by the action of both endogenous microbial enzymes and muscle enzymes [4,5]. The formation of end products originated by the changes of lipids, proteins, and fermentable sugars (i.e., glucose or fructose, added by the manufacturer) have a great impact on the quality characteristics of the product [6]. Fermented meats are included in the human diet as important constituents due to their nutritional value, shelf life, and special sensorial characteristics. Dry fermented sausages are traditional ready-to-eat meat products widely consumed in Europe [7–9]. The characteristic flavor and taste that make them unique are considered the most relevant attributes that strongly influence consumer acceptability and preference [10,11]. Lactic acid bacteria (LAB) are the most active microorganisms responsible for acidification during fermentation, resulting in the development of the desirable flavor, taste, color, and texture of the products. They produce lactic acid and bacteriocins that prevent the growth of spoilage and pathogenic microorganisms. Therefore, there is an enhancement in the stability, safety, and shelf-life of the sausages [6,11–13].

The main biochemical changes originating during the ripening of the dry-fermented sausages are lipolysis and proteolysis. During the lipid oxidation, the intramuscular phospholipids undergo significant changes generating polyunsaturated fatty acids such as linoleic, linolenic, and arachidonic acids [14]. The muscle peptidases (principally cathepsins) initiate the breakdown of the proteins along the process, whereas microbial enzymes exert their function in the final stages of aging [6,8]. Consequently, a substantial amount of low-molecular-weight compounds such as peptides and amino acids is released. The compounds produced by the lipolysis and proteolysis are intimately related to the organoleptic properties (aroma, taste, and flavor) and other diverse functionalities of the final dry-fermented product [13].

The growing interest in promoting and enhancing human health has increased the efforts of the food industry to search for functional ingredients [15]. Plants generally contain alkaloids, flavonoids, polyphenols, saponins, and tannins that influence animals due to their different functions and benefits [16]. The incorporation of these natural origin ingredients into meat products has gained attention during the last years since they are a source of bioactive compounds [17]. Another strategy to improve the nutritional value of meat products is to eliminate or reduce synthetic additives such as nitrogen compounds. This is related to their harmfulness. Breda et al. [18] indicated the carcinogenic effect of nitrite, pointing to its reactions with N-nitroso compound precursors in the gastrointestinal tract, thereby subsequently forming potentially carcinogenic compounds.

Nigella sativa, commonly known as black cumin, has been widely employed for centuries for culinary and pharmaceutical purposes throughout the world [19,20]. Seeds are highly valuable due to their content of bioactive compounds with antimicrobial, antioxidant, antifungal, and anti-inflammatory properties [19,21]. The most active constituent present in seeds is thymoquinone. Moreover, they also contain other active compounds such as carvacrol, p-cymene, 4-terpineol, trans-anethol, dithymoquinone, and thymohydroquinone, among others. In addition, they present amino acids, fats, reducing sugars, sterols, quinones, and essential minerals including calcium, copper, iron, and phosphorus and vitamins such as folic acid, niacin, pyridoxine, and thiamin [17,21–23].

Chia (*Salvia hispanica*) seeds have been popularly used as ingredients in healthy foods due to their high levels of dietary fiber and low carbohydrates [24,25]. These pseudocereals are characterized by their high concentration of essential polyunsaturated fatty acids such as α -linolenic acid (ω -3) and linoleic acid (ω -6). They contain a great number of antioxidants with strong activity such as flavonoids (myricetin, kaempferol, and quercetin), tocopherols, polyphenols, caffeic, cinnamic, and chlorogenic acids [26–29]. The functional seeds mentioned above offer benefits beyond their nutritional value as they exert beneficial properties for human health. They are suitable candidates as food ingredients in meat

products. There are some studies about the addition of chia seeds into meat products such as camel burgers [29] and frankfurters [25].

Therefore, the objective of the study is to compare the effects of *Salvia hispanica* seed and *Nigella sativa* seed in traditionally produced dry fermented sausages with reduced nitrites on the changes in physicochemical parameters (water content, pH, and water activity), color parameters, the content of nitrosopigments, and microbial counts throughout the production process. Additionally, at the end of the processing, the content of biogenic amines was determined.

2. Material and Methods

2.1. The Experimental Material Preparation

The study was performed in the Department of Meat Technology and Food Quality (University of Life Sciences in Lublin, Poland) in semi-technical conditions. Five different sample groups of dry fermented sausages were produced with reduced sodium nitrite addition (50 mg kg^{-1}) in relation to the permitted amount in accordance with the Commission Regulation (EU) No. 1129/2011 [30]. The amount of nitrite permitted for use in cured meat products is currently 150 mg kg^{-1} . Products were made with two replications. Dry fermented meat products were made using ham muscles from Polish large white purebred fatteners obtained from a local slaughterhouse at 48 h postmortem. The raw material was delivered in cooling conditions to the laboratory. Then, the meat was minced through a 10 mm grinding plate using the universal machine type KU2-3EK (Mesko-AGD, Skarżysko-Kamienna, Poland). To each formulation, 0.6% of glucose and 2.8% of curing mixture (Sea salt + Sodium nitrite) were added. The composition of the mixture and the amount of its application ensured the presence of sodium nitrite in the stuffing in the amount of 50 mg kg^{-1} . Grounded *Salvia hispanica* (Chia) seed and *Nigella sativa* (Black Cumin) seed were added into some samples (SCh1, SCh2, SBC1, and SBC2) in the amounts of 1% and 2%. The seeds were ground just before use using a knife mill (Bosch TSM6A017C) for particles less than 0.3 mm in diameter. *Salvia hispanica* seed and *Nigella sativa* seed were purchased in the local market. The groups of dry fermented sausages were presented in Figure 1. After mixing all ingredients using the universal machine type KU2-3EK (Mesko-AGD, Skarżysko-Kamienna, Poland) with an attached R4 type mixer (100 rpm, 3 min), the raw batters were stuffed into fibrous casings ($\varnothing 65 \text{ mm}$; Viskase, Chicago, USA) using a vertical manual sausage stuffer. Sausages of about 400 g were prepared. In the next step, sausage samples have been placed in fermentation chambers (ITALFROST-DE RIGO-GS, Pszczyna, Poland) for 30 days under controlled humidity and temperature conditions. Production conditions are given in Figure 1. Samples of each batch were taken at 1, 10, 20, and 30 days of the production.

The physicochemical parameters (water content, pH, and water activity), instrumental color, the content of nitrosopigments, and microbial counts (content of LAB and Enterobacteriaceae) were measured in stuffing (Day 1), 10, 20, and 30 days of the production. At 30 days, the content of biogenic amines and the content of *Listeria monocytogenes* were additionally made. The treatments were replicated twice by producing two different batches. Each sample was analyzed in triplicate.

Figure 1. The groups of dry fermented sausages and production conditions.

2.2. The Physicochemical Parameters (WC-Water Content, pH, and Water Activity)

The water content (WC) was determined according to PN ISO 1442:2000 [31]. A digital pH meter CPC-501 (Elmetron, Zabrze, Poland) equipped with a temperature sensor and pH electrode (ERH-111, Hydromet, Gliwice, Poland) was used to measure pH of the samples. The water activity (a_w) was measured using a water activity analyzer (Novasina AG, Lachen, Switzerland), which gives temperature-controlled measurements. Measurements were made at a temperature of 20 °C. Analyzer had been calibrated with Novasina SAL-T humidity standards (33%, 75%, 84%, and 90% relative humidity).

2.3. Lipid Oxidation Analyzes

Lipid oxidation in the sausages was assessed by measuring the amount of thiobarbituric acid reactive substances (TBARS) according to the procedure described by Pikul et al. [32]. The values were expressed as mg of malondialdehyde (MDA) per kilogram of sample.

2.4. Instrumental Color Measurement and Nitrosylmyoglobin Content

Color parameters were determined on the cross-section just after the sample (at least 20 mm thick) was cut [33]. Color parameters (L^* , a^* , b^*) were measured using an X-Rite 8200 colorimeter (X-Rite, Inc., Michigan, USA). Before use, the colorimeter was calibrated using the black glasses and white tiles provided. Samples were analyzed directly over the 12 mm aperture, with D65 illumination configurations and 10 viewing angles. The color difference (ΔE) between control and test samples during storage was calculated according to AMSA [33] using the following formula:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

In the interpretation of the results, it was assumed that when $0 < \Delta E < 1$, the observer does not notice the difference; when $1 < \Delta E < 2$, only an experienced observer may notice the difference; when $2 < \Delta E < 3.5$, an unexperienced observer also notices the difference; when $3.5 < \Delta E < 5$, a clear difference in color is noticed; and when $5 < \Delta E$, an observer notices two different colors [34].

The nitrosylmyoglobin content were determined according to the procedure proposed by Hornsey [35]. The amount of nitrosylmyoglobin was calculated according to Hornsey [35] and expressed in mg kg^{-1} .

2.5. Microbiological Analyzes

The microbiological analyses included the number of *lactic acid bacteria* (LAB) and the number of *Enterobacteriaceae* (EB) bacteria. They were made using the TEMPO[®] LAB automated microbial counting system (Biomérieux, TEMPO[®] System, Marcy l'Etoile, France). For microbiological determinations, the original TEMPO[®] tests were used to determine the number of lactic acid bacteria (TEMPO LAB) and the number of Enterobacteriaceae (TEMPO EB) in food products. The incubation conditions used for the TEMPO LAB and TEMPO EB tests include: incubation time (h)—40–48 and 22–27; temperature of incubation (°C)—37 and 35, respectively. The results are expressed as a logarithm colony-forming unit per gram of products ($\log \text{CFU g}^{-1}$). *L. monocytogenes* detection was carried out at Agrolab Polska Sp. z o. o. (Dęblin, Poland) according to ISO:11290-2:1998 [36].

2.6. Biogenic Amines (BAs) Determination

Biogenic amines (BAs) were determined in dry fermented sausages samples after extraction using 10% trichloroacetic acid (TCA). The analysis of BAs was performed using an AAA 500 amino acid analyzer (Ingos, Praha, Czech Republic), equipped with an Ostion LG AAA8 ion-exchange column ($3.6 \times 100 \mu\text{m}$, $8 \mu\text{m}$). Standards of BAs with a purity of 99% were obtained from Sigma-Aldrich (St. Louis, MO, USA). Solutions of BAs were prepared with a dilution buffer composed of 1.5 mM NaN_3 , 197 mM NaCl , and 73 mM citric acid in Milli-Q water. The system consisted of a filling chromatographic column and steel pre-column, two chromatographic pumps for transport of elution buffers and derivatization reagent, a cooled carousel for Eppendorf tubes, a dosing valve, a heat reactor, a Vis detector, and a cooled chamber for derivatization reagent. The volume of the injected sample was 100 μL . The reactor temperature was set to 120 °C. The content of the BA (tyramine, putrescine, cadaverine, spermidine, agmatine, and spermine) was determined with a reference to the amine standards, which were supplied by Ingos, Czech Republic. The BA concentrations were reported as mg g^{-1} of product.

2.7. Statistical Analysis

The collected data were analyzed using Statistica version 13.3 software (Dell Inc., Round Rock, TX, USA). The results were expressed as mean $\pm$ standard deviations. The data normality and homogeneity were analyzed using a Shapiro–Wilk test and a Levene's test. Effects between categorical factors (day and type of sausage) and variables between subgroups were analyzed using factorial ANOVA. The homogeneous groups are identified using the post hoc HSD Tukey's test. Additional information was obtained using the principal component analysis (PCA) method and the dendrite method for cluster analysis. The dataset was treated by Ward's method of linkage with squared Euclidean distance as a measure of similarity for multivariate analysis.

3. Results

3.1. The Results of Physicochemical Parameters (Water Content, pH, and Water Activity)

During the dry-curing process, a progressive diminution of water content was observed due to the water losses in all sausages (Table 1). No significant differences were

observed among batches, so the gradual losses were similar in all cases. On the last day of the study, the sausages lost half of their water content, from an average of 66% on Day 1 to 33% on Day 30. On the first day of analysis, all batches registered the highest pH values except the control, where the highest was at Day 30 (5.92) (Table 1). Moreover, the pH did not vary among the samples on the first day (5.69–5.78). The pH decreased up to 20 days of ripening, reaching the lowest values on the same day. However, on Day 30, there was a slight increase. It should be noted that the control sausages showed the highest pH on the rest of the sampling days (5.56, 5.27, and 5.92 at 10, 20, and 30 days, respectively). Conversely, the sausages with chia seeds were characterized by the lowest pH.

Table 1. The evolution of physicochemical parameters during the manufacturing process of dry fermented sausages.

Parameter	Treatment	Processing Day			
		Day 1	Day 10	Day 20	Day 30
Water content (%)	SK	67.09 ± 0.70 ^{aC}	49.66 ± 2.34 ^{aB}	36.28 ± 3.02 ^{aA}	32.87 ± 1.73 ^{aA}
	SCH1	66.33 ± 1.37 ^{aC}	50.48 ± 2.22 ^{aB}	35.72 ± 1.39 ^{aA}	33.44 ± 1.11 ^{aA}
	SCH2	64.41 ± 1.04 ^{aC}	48.65 ± 1.50 ^{aB}	34.21 ± 0.33 ^{aA}	32.93 ± 1.55 ^{aA}
	SBC1	65.37 ± 0.84 ^{aC}	50.06 ± 1.40 ^{aB}	36.61 ± 0.85 ^{aA}	34.33 ± 0.96 ^{aA}
	SBC2	65.69 ± 1.86 ^{aC}	50.01 ± 0.72 ^{aB}	34.75 ± 0.35 ^{aA}	32.36 ± 1.12 ^{aA}
pH	SK	5.75 ± 0.03 ^{aC}	5.56 ± 0.03 ^{dB}	5.27 ± 0.08 ^{dA}	5.92 ± 0.04 ^{cD}
	SCH1	5.78 ± 0.01 ^{aD}	4.92 ± 0.02 ^{bB}	4.72 ± 0.03 ^{abA}	5.29 ± 0.03 ^{aC}
	SCH2	5.69 ± 0.01 ^{aD}	4.81 ± 0.05 ^{aB}	4.62 ± 0.01 ^{aA}	5.21 ± 0.02 ^{aC}
	SBC1	5.72 ± 0.05 ^{aD}	5.04 ± 0.02 ^{cB}	4.89 ± 0.06 ^{bA}	5.49 ± 0.04 ^{bC}
	SBC2	5.74 ± 0.05 ^{aD}	4.94 ± 0.03 ^{bcB}	4.81 ± 0.07 ^{bcA}	5.47 ± 0.11 ^{bC}
Water activity	SK	0.968 ± 0.005 ^{aD}	0.913 ± 0.010 ^{aC}	0.829 ± 0.017 ^{aB}	0.801 ± 0.010 ^{aA}
	SCH1	0.973 ± 0.003 ^{aD}	0.917 ± 0.006 ^{aC}	0.835 ± 0.010 ^{aB}	0.802 ± 0.001 ^{aA}
	SCH2	0.972 ± 0.006 ^{aD}	0.915 ± 0.002 ^{aC}	0.830 ± 0.003 ^{aB}	0.801 ± 0.005 ^{aA}
	SBC1	0.968 ± 0.005 ^{aD}	0.910 ± 0.005 ^{aC}	0.830 ± 0.005 ^{aB}	0.813 ± 0.002 ^{aA}
	SBC2	0.969 ± 0.004 ^{aD}	0.910 ± 0.001 ^{aC}	0.831 ± 0.001 ^{aB}	0.798 ± 0.012 ^{aA}

SK—control sample; SCH1—sample with 1% addition of chia seed; SCH2—sample with 2% addition of chia seed; SBC1—sample with 1% addition of black cumin seed; SBC2—sample with 2% addition of black cumin seed; means with the same letter a–d do not differ significantly ($p > 0.05$) within the variable in the same day (column); means with the same cover letter A–D do not differ significantly ($p > 0.05$) within the same variant of variable in different day (row).

As in the case of water content, the water activity in the samples decreased significantly throughout 30 days (Table 1). There were no significant differences within the different sausages regardless of the day of the study. The samples on Day 1 were characterized by water activity in the range of 0.968–0.973, while on Day 30, they were characterized by water activity in the range of 0.798–0.813.

3.2. The TBARS Results

The TBARS results are reported in Table 2. During the first 10 days, no differences were observed between the sausage samples. In addition, the control sample and sample with black cumin addition suffered similar oxidation throughout the processing time. However, after Day 20, the samples with chia showed greater oxidative degradation than the other batches. With higher chia percentage, the oxidation was higher (2.24 (2%) vs. 1.38 (1%) and 2.53 (2%) vs. 1.31 (1%) at 20 and 30 days, respectively). Solely the samples with chia underwent lipid oxidation over time. Both control and black cumin samples remained at the same level of oxidation during the whole study.

Table 2. The evolution of color parameters, nitrosylmyoglobin, and TBARS content during the manufacturing process of dry fermented sausages.

Parameter	Treatment	Processing Day			
		Day 1	Day 10	Day 20	Day 30
L* color parameter	SK	49.24 ± 1.73 ^{aB}	45.58 ± 1.88 ^{aB}	37.44 ± 1.56 ^{aA}	35.61 ± 3.87 ^{aA}
	SCH1	47.43 ± 1.53 ^{aAB}	51.99 ± 2.22 ^{bB}	43.41 ± 1.29 ^{abA}	43.08 ± 4.72 ^{bA}
	SCH2	47.95 ± 2.34 ^{aAB}	51.74 ± 0.88 ^{bB}	48.31 ± 2.64 ^{bAB}	43.01 ± 3.26 ^{bA}
	SBC1	45.17 ± 1.87 ^{aB}	48.27 ± 2.69 ^{abB}	42.96 ± 3.41 ^{abB}	35.76 ± 1.74 ^{aA}
	SBC2	44.61 ± 1.80 ^{aB}	48.32 ± 1.16 ^{abB}	42.40 ± 6.51 ^{abAB}	37.07 ± 3.59 ^{abA}
a* color parameter	SK	7.78 ± 1.05 ^{abA}	9.34 ± 0.84 ^{bA}	9.32 ± 2.75 ^{bA}	8.03 ± 1.55 ^{bA}
	SCH1	8.08 ± 0.80 ^{bA}	9.85 ± 1.11 ^{bA}	9.97 ± 0.57 ^{bA}	9.06 ± 1.16 ^{bA}
	SCH2	9.08 ± 1.35 ^{bA}	10.09 ± 0.75 ^{bA}	7.73 ± 0.54 ^{bA}	8.18 ± 0.61 ^{bA}
	SBC1	5.85 ± 0.96 ^{aA}	7.69 ± 0.80 ^{abA}	5.73 ± 0.81 ^{abA}	6.61 ± 1.17 ^{abA}
	SBC2	5.55 ± 0.69 ^{aA}	6.47 ± 0.10 ^{aA}	4.42 ± 0.40 ^{aA}	4.45 ± 1.02 ^{aA}
b* color parameter	SK	9.27 ± 0.43 ^{abC}	6.98 ± 0.55 ^{abB}	6.77 ± 1.14 ^{bB}	5.01 ± 0.84 ^{abA}
	SCH1	10.13 ± 0.78 ^{bC}	8.08 ± 0.60 ^{bcB}	7.00 ± 1.53 ^{bAB}	5.48 ± 1.07 ^{bA}
	SCH2	10.64 ± 0.46 ^{bC}	9.07 ± 0.46 ^{cB}	5.90 ± 0.60 ^{bA}	6.05 ± 1.32 ^{bA}
	SBC1	7.95 ± 0.75 ^{aC}	6.38 ± 0.74 ^{aB}	3.70 ± 0.31 ^{aA}	4.29 ± 0.91 ^{aA}
	SBC2	7.55 ± 0.51 ^{aC}	6.22 ± 0.77 ^{aB}	3.46 ± 0.20 ^{aA}	3.91 ± 1.07 ^{aA}
ΔE	SK				
	SCH1	3.14 ± 1.12 ^a	7.30 ± 2.35 ^a	6.91 ± 2.07 ^a	9.85 ± 2.13 ^b
	SCH2	3.68 ± 1.70 ^a	6.66 ± 2.17 ^a	11.37 ± 2.64 ^a	7.79 ± 4.44 ^{ab}
	SBC1	5.21 ± 2.26 ^a	4.35 ± 2.03 ^a	8.30 ± 2.46 ^a	4.67 ± 1.73 ^a
	SBC2	5.75 ± 1.14 ^a	4.69 ± 1.63 ^a	10.63 ± 2.24 ^a	5.54 ± 2.63 ^{ab}
Nitrosylmyoglobin (mg kg ^{−1})	SK	11.74 ± 3.56 ^{aA}	13.97 ± 0.85 ^{aA}	25.81 ± 3.97 ^{aB}	28.56 ± 5.04 ^{aB}
	SCH1	14.93 ± 2.20 ^{aA}	38.47 ± 0.54 ^{bB}	48.57 ± 2.54 ^{bcC}	56.07 ± 4.65 ^{bcC}
	SCH2	15.32 ± 1.19 ^{aA}	40.07 ± 1.01 ^{bcB}	42.68 ± 6.51 ^{bcC}	33.54 ± 12.61 ^{aB}
	SBC1	16.57 ± 1.77 ^{aA}	39.34 ± 1.66 ^{bB}	52.49 ± 3.59 ^{cC}	58.24 ± 1.74 ^{bcC}
	SBC2	18.17 ± 1.65 ^{aA}	44.03 ± 1.80 ^{bB}	62.35 ± 2.72 ^{dC}	66.55 ± 2.63 ^{cC}
TBARS (mg kg ^{−1})	SK	0.64 ± 0.05 ^{aA}	0.77 ± 0.22 ^{aA}	0.87 ± 0.29 ^{aA}	0.95 ± 0.09 ^{aA}
	SCH1	0.68 ± 0.06 ^{aA}	0.77 ± 0.14 ^{aAB}	1.38 ± 0.10 ^{aC}	1.31 ± 0.14 ^{aBC}
	SCH2	0.70 ± 0.08 ^{aA}	0.98 ± 0.18 ^{aA}	2.24 ± 0.89 ^{bB}	2.53 ± 1.00 ^{bB}
	SBC1	0.74 ± 0.08 ^{aA}	0.90 ± 0.14 ^{aA}	1.15 ± 0.08 ^{aA}	1.03 ± 0.08 ^{aA}
	SBC2	0.79 ± 0.06 ^{aA}	1.11 ± 0.19 ^{aA}	1.11 ± 0.04 ^{aA}	1.19 ± 0.11 ^{aA}

SK—control sample; SCH1—sample with 1% addition of chia seed; SCH2—sample with 2% addition of chia seed; SBC1—sample with 1% addition of black cumin seed; SBC2—sample with 2% addition of black cumin seed; means with the same letter a–d do not differ significantly ($p > 0.05$) within the variable in the same day (column); means with the same cover letter A–C do not differ significantly ($p > 0.05$) within the same variant of variable in different day (row).

3.3. The Results of Instrumental Color Measurement and Nitrosylmyoglobin Content

The results of color parameters (L*, a*, b*) are also given in Table 2. In general, the lightness (L*) experimented a reduction from Day 1 to 30 in all samples (49.24 vs. 35.61 (SK), 47.43 vs. 43.08 (SCH1), 47.95 vs. 43.01 (SCH2), 45.17 vs. 35.76 (SB1), and 4.61 vs. 37.07 (SBC2)). Nevertheless, the highest values were reached at Day 10 for SCH1 (51.99), SCH2 (51.74), SBC1 (48.27), and SBC2 (48.32), and at Day 1 for SK (49.24). No differences in L*

were observed among the different samples during the first day of the ripening process, for which values ranged from 44.61 to 49.24. However, on the rest of the days, SCH sausages had higher values than SBC sausages. In turn, SBC was characterized by the slightly higher lightness at 10 and 20 days compared to the SK sample.

The same redness values (a^*) was maintained throughout the time in all batches (SK (7.78–9.34), SCH1 (8.08–9.97), SCH2 (7.73–10.09), SBC1 (5.73–7.69), and SBC2 (4.42–6.47)). The sausages with the addition of black cumin presented the lowest a^* at any of the days, whereas the sausages elaborated with chia seeds had the greatest redness value. Even the control sample had similar value of a^* parameter compared to the sausages with chia seeds.

Regarding yellowness (b^*), this parameter decreased progressively with time in all samples, except for SCH2, SBC1, and SBC2, where they showed the same degree of b^* from days 20 to 30 (SK (9.27–5.01), SCH1 (10.13–5.48), SCH2 (10.64–6.05), SBC1 (7.95–4.29), and SBC2 (7.55–3.91)). In case of the b^* parameter, the same trend as for a^* parameter was observed. The SBC samples showed the lowest b^* values, while samples with chia seed were characterized by the highest b^* color parameter values.

The color differences (ΔE) between control and test samples showed that the greatest color changes took place at Day 20. At this day, the color changes for the samples ranged from 6.91 to 11.37. The greatest color changes were shown between the control and SCH2 sample. The values ΔE higher than 5 mean that an observer notices two different colors for samples.

The content of nitrosylmyoglobin increased with time, leading to the greatest amount on the last day (Table 2) with the exception of the SCH2 samples. The samples with chia seed were characterized by the highest content of nitrosylmyoglobin at Day 20 (45.68 mg kg⁻¹). No differences among the sausages were observed during the first day. SK sample showed the lowest content of this compound on any of the days studied (11.74, 13.97, 25.81, and 28.56 mg kg⁻¹ at 1, 10, 20, and 30 days), whereas the SBC samples showed the highest values. Slightly more concentration was reached in the sausages with 2% of black cumin seeds (18.17 vs. 16.57, 44.03 vs. 39.34, 62.35 vs. 52.49, and 66.55 vs. 58.24 mg kg⁻¹ at 1, 10, 20, and 30 days).

3.4. The Results of Microbiological Analyzes

Regarding the changes on microbial growth of the *Enterobacteriaceae* (Figure 2), from Day 1 onwards, the Gram-negative bacteria decreased, reaching the minimum levels at Day 10 (1.51, 1.04, 0.66, 1.11, and 0.70 log CFU g⁻¹ for SK, SCH1, SCH2, SBC1, and SBC2, respectively). It can be highlighted that the control samples presented the highest number of colonies on any of the days, exceeding 5 log CFU g⁻¹ on day 0 and almost 3 log CFU g⁻¹ on Day 30. On the other hand, the addition of a higher percentage of seeds resulted in a decrease of *Enterobacteriaceae*, achieving the lowest values on SCH2 sausages at 10 and 20 days (0.66 (2%) vs. 1.03 (1%) and 2.11 (2%) vs. 2.95 (1%) log CFU g⁻¹) and SBC2 (1.32 (2%) vs. 1.83 (1%) log CFU g⁻¹) at 30 days. Furthermore, comparing the different seeds with the same percentage of addition, chia seeds provided lower values in all cases, excepting the 2% addition at Day 30, where black cumin achieved better results.

Concerning the lactic acid bacteria growth, the proliferation increased ongoing the fermentation process, where the amount surpassed the 7 log CFU g⁻¹ on days 10, 20, and 30 in all cases except for the control, which had the same amount of LAB on the first and last day of ripening. Due to food safety, we also determined the content of *L. monocytogenes*. None of samples contained this microorganism (<10 CFU g⁻¹).

Parameter	Treatment	Day 30
<i>L. monocytogenes</i> [CFU g ⁻¹]	SK	<10
	SCH1	<10
	SCH2	<10
	SBC1	<10
	SBC2	<10

Figure 2. The results of microbiological analyzes during the manufacturing process of dry fermented sausages. SK—control sample; SCH1—sample with 1% addition of chia seed; SCH2—sample with 2% addition of chia seed; SBC1—sample with 1% addition of black cumin seed; SBC2—sample with 2% addition of black cumin seed.

3.5. Results of Cluster and PCA Analysis

Figure 3 shows a cluster analysis dendrogram that takes into account all types of samples (factors are: type of supplement, its level, and day). In the course of the analysis, a

group of six samples (the first from the top) was distinguished, especially from the study on Day 1. SK sample at Day 20 (SK_20) slightly differs from the others in this group. The remaining trials (10, 20, and 30 days) form a separate group. The presented data clearly indicate that the storage time modifies the properties of the product.

Figure 3. Cluster analysis dendrogram. SK—control sample; SCH1—sample with 1% addition of chia seed; SCH2—sample with 2% addition of chia seed; SBC1—sample with 1% addition of black cumin seed; SBC2—sample with 2% addition of black cumin seed.

Figure 4 shows the loading plot of the measurements of sausages quality. In the case of the samples with chia seed, the first component (p1) is negatively affected by pH a^* and positively affected by L^* . On the other hand, the second component (p2) is determined by the negative influence of NOMb, TBARS, and positive- b^* , WC, and a_w . There is a positive correlation for the parameters NOMb and TBARS, as well as WC, and w and b^* . A negative correlation was also observed between NOMb and the parameters describing water availability (a_w and WC). A similar relationship applies to TBARS and pH. Interestingly, there is also a weak correlation between the color parameters (a^* , L^*) and the parameters describing water availability, i.e., a_w and WC. Similarly, low correlation applies to pH and parameters such as L^* , b^* , and WC. In the case of samples with black cumin, the first component is influenced most positively by pH, a^* color parameter, and negatively by L^* parameter. On the other hand, the second component is determined by the negative influence of NOMb and TBARS and the positive influence of b^* , WC, and a_w . There is a clear positive correlation for the NOMb and TBARS parameters, as well as for a^* and pH, and WC and a_w . As expected, NOMb and TBARS are related to oxidation, and water activity is determined by WC. The pH value influences the redness (a^*). It is also worth noting the negative correlation between NOMb and a^* , as well as TBARS and pH. It is also interesting that there is no significant correlation between the color parameters and the parameters describing water availability, i.e., a_w and WC.

Figure 4. Loadings for the two principal components. (a) for samples with chia seed addition; (b) for samples with black cumin seed addition.

3.6. Biogenic Amines Content

Table 3 reports the amount in mg g^{-1} of the identified amines at the last day of the experiment. The most abundant biogenic amines present in all sausages were the tyramine and putrescine. Moreover, the samples with seeds addition had a higher concentration of BAs compared to the control sausage. The highest concentration of these organic compounds was achieved with the incorporation of chia seeds followed by the black cumin. In addition, it can be seen that with a higher percentage of chia seeds added, the amount of tyramine slightly increased (0.194 and 0.223 mg g^{-1} for the additions of 1% and 2%, respectively). However, the opposite behavior was observed in the spermine where the control samples had the maximum levels (0.089 mg g^{-1}) and the black cumin the lowest (0.060 (1%) and 0.059 (2%)). No significant differences were found in cadaverine, where the values ranged from 0.099 – 0.148 mg g^{-1} . All sausages showed similar amounts of spermidine except for SCH2, which was characterized by higher cadaverine content

(0.016 mg g⁻¹). Regarding the agmatine, a natural aliphatic polyamine produced from arginine, it was only detected in control samples at a concentration of 0.105 mg g⁻¹.

Table 3. The biogenic amines content at 30 day of manufacturing process of dry fermented sausages [mg g⁻¹].

	Tyramine	Putrescine	Cadaverine	Spermidine	Agmatine	Spermine
SK	0.094 ± 0.066 ^a	0.048 ± 0.023 ^a	0.099 ± 0.051 ^a	0.010 ± 0.003 ^a	0.105 ± 0.012	0.089 ± 0.036 ^c
SCH1	0.194 ± 0.080 ^{ab}	0.114 ± 0.058 ^c	0.103 ± 0.107 ^a	0.010 ± 0.005 ^a	nd	0.055 ± 0.028 ^{abc}
SCH2	0.223 ± 0.053 ^b	0.147 ± 0.009 ^c	0.144 ± 0.043 ^a	0.016 ± 0.001 ^b	nd	0.070 ± 0.003 ^{bc}
SBC1	0.147 ± 0.028 ^{ab}	0.104 ± 0.005 ^b	0.120 ± 0.046 ^a	1.010 ± 0.001 ^a	nd	0.060 ± 0.003 ^{ab}
SBC2	0.166 ± 0.056 ^{ab}	0.108 ± 0.004 ^b	0.148 ± 0.066 ^a	0.011 ± 0.001 ^a	nd	0.059 ± 0.003 ^a

nd—not detected; SK—control sample; SCH1—sample with 1% addition of chia seed; SCH2—sample with 2% addition of chia seed; SBC1—sample with 1% addition of black cumin seed; SBC2—sample with 2% addition of black cumin seed; means with the same letter a–c do not differ significantly ($p > 0.05$) within the variable (column).

4. Discussion

4.1. Physicochemical Parameters (Water Content, pH, Water Activity)

The addition of chia or black cumin did not exert any effect on water content or water activity changes since no significant differences were found compared to the control. Our data differed from other authors whose results revealed that the incorporation of chia seeds into chicken sausages facilitates the moisture reduction [37]. In addition, they observed that with a higher percentage of seeds, the moisture is lower at day 0 (63.24%, 62.57%, and 61.32% for 2%, 4%, and 6% of chia seeds). The same trend was observed in pork dry-cured sausages [38] at Day 3 of storage, where the 6% chia sausages showed a lesser percentage of moisture than the control samples (33.13% vs 42.48%). As in our experiment, they also found a decrease in water activity with time. The amount of chia did not affect the a_w values. There were no differences in water content in another study of frankfurters with 3% chia seeds concerning the control samples [24]. The pH decreased in all the samples during the ripening process. This tendency could be explained due to the growth of LAB. The main function of this bacteria is to reduce the pH through the production of lactic acid from the fermentation of sugars [39]. However, the production of acids also depends on the concentration and type of sugars added and the diameter of the sausages [40]. The lowest pH achieved with chia sausages on Day 20 of the production process is due to the highest proliferation of LAB in this type of sausages. In any cases, it has been reported that the incorporation of chia seeds lowered the pH levels in frankfurters after 14 and 21 days [24]. On the other hand, the introduction of chia seeds in pork dry-cured sausages did not modify the pH over time, though on Day 0, the pH was significantly lower than the control samples (5.63 vs. 5.78) [38].

4.2. Lipid Oxidation Analyzes

The TBARS method is commonly used as a marker of lipid peroxidation to control lipidic changes during the processing of fermented sausages. At the beginning of the study up to Day 20, there were no differences between the sausages on TBARS values. Moreover, TBARS values did not alter in both control and sausages with black cumin throughout the study. Thus, the incorporation of black cumin did not affect lipid peroxidation. The benefits of chia seeds, which are an excellent source of antioxidants, are well-known. However, the incorporation of chia seeds into dry fermented sausages led to a significant increase in malondialdehyde concentration after 20 days, pointing to these sausages as the most oxidized. Even in the sausages with higher chia content, the oxidation was greater. This could be explained due to the higher concentration of polyunsaturated fatty acids in chia. Additionally, malondialdehyde is mainly formed from linolenic acid oxidation, a compound present in chia seeds. Furthermore, aldehydes from sugars and phenolic compounds may interfere on TBARS [41]. Nonetheless, these findings are in contradiction

with other authors, who found that chia seeds retard oxidation. Specifically, sausages with 3% chia suffered less oxidation than the control (roughly 1.6 less) [24]. The same trend was observed in dry-cured sausages (around 1.2 times lower, Day 3) [38] as well as in camel burgers (around 1.3 times lower, Day 12) [28].

4.3. Instrumental Color Parameters and Nitrosylmyoglobin Content

Color is one of the foremost sensory attributes for meat and meat products consumers since it is associated with freshness and quality [42,43]. All sausages suffered a lightness and yellowness reduction from Day 1 to Day 30. On the last day, both the control sausage and the sausages with black cumin showed similar values of L* parameter, being significantly lower than the sausages with chia seed addition. The redness (a* parameter) remained invariable in every sample during the whole processing period. The control sausage and sausages with chia seed exhibited the highest b*, whereas the black cumin addition provided the lowest value. Our results differed from chia frankfurters [24], which presented lower a* and L* than control frankfurters at Day 21. Moreover, while L* decreased by the time, a* and b* showed similar values at Days 0 and 21 in the case of chia samples. During fermentation, the proliferation of Gram-positive microorganisms provide color stabilization due to their ability to form a nitrosylmyoglobin complex through the reaction of nitric oxide with myoglobin. This compound is responsible for the unique color in cured meat [44,45]. In our study, the nitrosylmyoglobin content recorded increased broadly along the time. Sausages with seeds added provided the greatest results, especially those with higher proportion of black cumin added. Łaskiewicz et al. [46] pointed out that the lactic acid bacteria contribute to the synthesis of nitrosyl pigments in cured poultry meat. Other researchers explained the same phenomenon [42,43]. Therefore, our results are well-correlated with the acid lactic bacteria since the largest values for nitrosylmyoglobin were observed in the sausages with the highest lactic acid bacteria content.

4.4. Microbiological Analyzes

The *Enterobacteriaceae* could have been derived from the raw tissues, contaminated in the slaughterhouses during the slaughtering and quartering of the animal. The *Enterobacteriaceae* counts decreased in all samples until Day 10 of ripening, increasing in the following 20 days before again decreasing until the end of the processing. Sausages with seed additions were characterized by a lower *Enterobacteriaceae* content in comparison to the control. This fact indicated that chia and black cumin had a positive effect on reducing the tested bacterial count. Studies by Duda-Chodak et al. [47] proved that kaempferol and quercetin as the main *Salvia hispanica* bioactives representatives could exert a strong and negative effect on the development of both opportunistic and truly pathogenic microorganisms, including *Enterobacteriaceae*. Traditionally, fermented sausages are manufactured using lactic acid bacteria and coagulase-negative staphylococci that are naturally present in meat. During the fermentation, there is an increase in the lactic acid bacteria count. As expected, the LAB count at the end of ripening was higher than on the first day, excluding the control, where the first and the last day showed the same growth. This fact suggests that the addition of chia or black cumin seed enhances the proliferation of the LAB. LAB are safe microorganisms; they can inhibit pathogens, producing a wide range of inhibitory compounds such as bacteriocins, carbon dioxide, diacetyl, ethanol, hydrogen peroxide, and lactic acid [48]. The rapid growth of LAB is important to become dominant and competitive against foodborne pathogens such as *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes*, and *Staphylococcus aureus*. As mentioned before, the principal role of LAB is to acidify the matrix through lactic acid generated by the fermentation of carbohydrates [40]. One of the most frequently detected pathogens in dry fermented sausages is the *L. monocytogenes*. The optimal conditions for their growth are pH values between 6.0–8.0 and water activities above 0.92, though they can survive even at lower and higher pH (4.5–9.0). Thus, the drying step is crucial to reduce water activity and consequently minimize the survival of *L. monocytogenes* [49]. After 30 days, none of the sausages contained these

bacteria (<10 CFU g^{-1}). The reason is because after the ripening stage, the water activities of sausages ranged from 0.813–0.798, and pH was under 6.0. Furthermore, *L. monocytogenes* could not compete due to the high population of LAB [40,49].

4.5. Biogenic Amines Content

Fermented and protein-rich foods are prone to forming toxic nitrogenous bases such as biogenic amines. These low-molecular-weight compounds are generated due to the microbial decarboxylation of amino acids through substrate-specific decarboxylase enzymes [50,51]. Some authors reported that tyramine, putrescine, and cadaverine are the predominant biogenic amines in fermented foods [51,52]. As we could see, these compounds were those with the highest concentration in experimental sausages ranging from 0.094–0.223 mg g^{-1} (tyramine), 0.048–0.147 mg g^{-1} (putrescine), and 0.099–0.148 mg g^{-1} (cadaverine). Among these biogenic amines, tyramine has attracted special consideration due to its potential health risks at high levels. It is synthesized by the fermentative microbial population of lactic acid bacteria [53]. For this reason, sausage samples had a high level of tyramine since the growth of lactic acid bacteria increased considerably during the ripening process. Our results are well-correlated with the microbial of lactic acid bacteria since the control (SK) had both the lowest concentration of tyramine and the lowest growth of lactic acid bacteria. LAB and *Enterobacteriaceae* are capable of forming biogenic amines [54], though the LAB are the main producers [55]. Therefore, samples with a higher bacterial population are expected to contain more amines. However, the aliphatic diamines cadaverine and putrescine could potentiate the adverse effects of tyramine [56]. These substances are formed during food processing and storage by the action of bacteria. As BAs are indicators of the microbiological quality of food, their identification and quantification are important [57]. Agmatine can be metabolized to putrescine, the spermine and spermidine precursor [58]. The aminogenesis is strongly influenced by many factors, though the most relevant is the microbiological quality of raw material (decarboxylase activity is mainly attributed to enterobacteriaceae, pseudomonadaceae, micrococcaceae, lactic acid bacteria, etc.), which can vary in each production batch. Furthermore, the characteristics of the raw material (composition, ion strength, pH, etc.), ingredients, additives, and the processing and storage conditions are also crucial factors that produce variations in the content of the different biogenic amines. Perhaps, due to the raw meat employed, the biogenic content in all sausages was quite higher compared with other studies [59]. Although they are considered a food hazard, there is no threshold for biogenic amines in European legislation, except for histamine in fish [60]. Biogenic amines are thermostable; therefore, after their formation, their elimination is quite difficult. Hence, it is critical to avoid their presence by employing appropriate materials and conditions to ensure food quality [52,61,62]. In addition, the fast acidification caused by negative amino decarboxylase cultures reduces the development of biogenic amines in sausages [48].

5. Conclusions

The incorporation of plant material, rich in bioactive compounds, in the preparation of fermented meat products is one of the strategies to develop innovative meat products with an improved nutritional profile. Chia and black cumin seed additions promoted changes in characteristics of traditional fermented sausages with reduced nitrites during 30 days of production. The addition of seeds caused a greater reduction in the pH of the sausages during the production process. The lowest pH and the highest LAB content were achieved with sausages with chia seed on the last day of the production process. Additionally, the largest values for nitrosylmyoglobin were observed in the sausages with the highest lactic acid bacteria content (samples with chia and black cumin seed addition), which confirms that the lactic acid bacteria contribute to the synthesis of nitrosyl pigments. Chia and black cumin seed incorporation in fermented sausages reduced *Enterobacteriaceae*; however, it affected higher concentration of BAs. Chia and black cumin appear to have a similar effect on the quality of fermented sausages. Generally, no significant differences were found in

the values of most of the parameters assessed for the samples with 1% and 2% addition of seeds. The obtained results indicate that this plant material can be used as a natural additive in the production of fermented sausages with improved nutritional value.

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