

Article

The Effect of Different Levels of Sodium Nitrate on the Physicochemical Parameters and Nutritional Value of Traditionally Produced Fermented Loins

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Abstract: This study evaluated the effect of sodium nitrate reduction on the following parameters: thiobarbituric acid reactive substances, the color parameters (CIE L* a* b*), total heme pigments, heme iron content and nutritional value related to N-nitrosamines content as well as nitrate and nitrite residues of traditionally produced fermented loins. Raw loins (*m. longissimus thoracis et lumborum*) and fermented products with different levels of nitrate added (0, 50, 100, 150 mg kg⁻¹) were tested during six months of vacuum storage. The reduction of nitrate did not lead to statistically significant changes in total pigment content as well as heme iron content in fermented loins at the end of processing and during storage. Water activity did not differ statistically significantly between the formulations. Fermented loins at the end of processing revealed residual sodium nitrite levels of <10 mg kg⁻¹, while the amount of nitrate residue depended on the level added during production, obtaining the highest value of 19.0 mg kg⁻¹ for the sample with the highest nitrate addition. The level of nitrosamines was <5 µg kg⁻¹ in all samples, which proves their chemical safety. In conclusion, the use of nitrate reduced to 50 mg kg⁻¹ in fermented loins allows to obtain a product with properties similar to the product with 150 mg kg⁻¹ of nitrate, especially in terms of its physicochemical properties and lipid oxidation.

Keywords: fermented loins; nitrate; nutritional value; N-nitrosamines



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1. Introduction

The use of nitrites and nitrates is a part of the meat processing tradition associated with their multidirectional impact on the quality of meat products including microbiological and chemical safety. The main purpose of using nitrites or nitrates is related to their preservative effect by inhibiting the growth of bacteria (especially *Clostridium botulinum* and spore germination). They also delay the progress of oxidative reactions in the main components of meat (proteins and lipids) and participates in the formation of the characteristic color (called the “cured meat” color) and taste of meat products [1]. When added to meat, the nitrates must first be reduced to nitrite in order to become active curing agents. The reduction of nitrates to nitrites occurs with the participation of bacteria possessing nitrate reductase activity [2]. The most known and most effective microorganisms in reducing nitrates are staphylococci and micrococci; however, lactic acid bacteria can also reduce nitrates to nitrites influencing the course of the fermentation process in meat products. Moreover, according to Hammes [2], nitrate reductase activity is normally present in animal physiology and the presence of microorganisms in this process is not essential. At the optimum pH of meat (5.6 to 5.8), nitrites decrease by conversion to the compounds that can act as oxidizing, reducing or nitrosylating agents (nitrous acid, nitric oxide and nitrates) [3]. The production of nitric oxide from nitrite is required for the reaction with myoglobin, the protein responsible for the color of the meat. The formation of nitrosyl myoglobin generates the cured meat product color. The effect of nitrates on aroma is also well-known. It is

related to the content of aldehyde compounds (producing green odor notes) formed during lipid oxidation reactions and key sulfur odor compounds responsible for meaty odors [4]. The literature also describes the mechanisms of the antioxidant properties of nitrites [5].

Despite the beneficial properties of nitrites and nitrates in meat products, the processing of meat using these additives is closely related to the harmful effects of high consumption of processed meat, as reported by the International Agency for Research on Cancer [6]. Their involvement in the formation of nitroso-compounds such as carcinogenic N-nitrosoamines is widely known [7]. N-nitrosamines can form through nitrosation reactions. The nitric oxide, formed by the reduction of nitrites, can react with secondary amines to form nitrosamines. Several conditions favor the formation of these compounds, such as acid pH with the amine protonated, a large amount of residual nitrite and a longer storage time [8]. The negative health effects of nitrates and nitrites are commonly described in the literature [9–11]. On the other hand, in recent years, various benefits of the supply of nitrates with the diet have been indicated, in particular on the function of the cardiovascular system [12,13]. These benefits are related to the supply of nitric oxide, which have a positive effect on the cardiovascular system. The role of nitrites/nitrates in this context is multidirectional; their protective effect in lowering blood pressure and inflammation, improving exercise capacity and mitochondrial function, lowering triglycerides and reducing heart attacks and strokes is indicated. Although the scientific evidence shows the positive effects of nitrates in our body, there is still huge pressure from consumers to choose nitrate-free meat products. On the other hand, despite the increased risk of cancer associated with eating large amounts of processed meat [6], the consumption and production of meat products worldwide is increasing due to the globalization [14].

According to Commission Regulation (EU) No. 1129/2011 [15], nitrates (designated as E251 and E 252, respectively) and nitrites (E249, E250) are included in the list of additives approved for use in meat processing. The amount of nitrite permitted for use in cured meat products is currently 150 mg kg^{-1} . Although the use of these additives is permitted in meat processing, consumer concern motivates researchers and the meat producers to produce meat products in line with the trend 'clean label' without using nitrates/nitrites [16]. In this context, some researchers undertake research on the effect of reducing the addition of nitrates on the quality of meat products, with particular emphasis on their shelf life [17,18]. Our previous studies [19] suggested that the use of nitrite in the amount lower than permitted by law allows to obtain a cooked meat product with properties similar to the product with 150 mg kg^{-1} of nitrite, especially in terms of physicochemical properties and lipid oxidation. However, the reduction of nitrates in traditionally produced fermented meat products carries the risk of a shortened shelf life. In this type of meat product, not subjected to heat treatment, nitrates play a special role, in particular due to their antioxidant properties and their participation in color formation. As shown by previous studies, the elimination or reduction of nitrate may affect the fermentation process during the production of meat products [20–22].

In this context, the aim of the present study was to determine the effect of sodium nitrate reduction to the level 100, 50 and 0 mg kg^{-1} on lipid oxidation measured as thiobarbituric acid reactive substances (TBARS), the color parameters (CIEL* a* b*) total heme pigments, heme iron content and nutritional value related to N-nitrosamines content as well as nitrate and nitrite residues of traditionally produced fermented loins during six months of vacuum storage. Based on previous research, it was hypothesized that reducing the nitrate addition by more than half will allow to obtain a fermented meat product with characteristics similar to the product with the traditional addition of nitrate.

2. Materials and Methods

2.1. Production of Fermented Loins

The raw materials for the production of experimental meat products were loins (*m. longissimus thoracis et lumborum*) from Polish large white purebred fatteners at 24 h after slaughter. The loins were obtained from a slaughterhouse located near Biłgoraj (in Eastern

Poland). The raw materials were delivered in hygienic and cooling conditions to the Department of Meat Technology and Food Quality of University of Life Sciences in Lublin. Then, they were stored at 4 ± 2 °C during 24 h. After a day had passed, the meat was prepared for processing to obtain fermented loins. Preparation included the cleaning of the surface of the fascia, removing the protruding parts of the muscle, and giving the portion with a similar weight (2.0 ± 0.5 kg). The loins prepared in this way were divided into parts of about 500 g. Prepared parts of meat have been processed using the following operations: (1) salting (2.8%) with sea salt or curing (2.8%) with a mixture containing sea salt (99.5%) and sodium nitrate(V) (0.5%) using a surface massage, and (2) fermentation and maturation in the fermentation chamber. The addition of glucose in the amount of 6 g kg^{-1} was used for all samples. Four different sample groups of fermented loins were produced with various percentages of sodium nitrate (0, 50, 100, and 150 mg kg^{-1}). Fermentation and maturation were carried out in a chamber under controlled conditions for 30 days under controlled humidity and temperature. After production, the finished products were packed in nylon-polyethylene bags ensuring vacuum conditions. The samples were stored at the temperature of 4 ± 2 °C until analysis. The first analyzes were made on the next day after packing the products (zero months), the next after one, four and six months of storage. The formulation of fermented loin treatment, production and storage conditions are given in the Table 1. The production and analysis of fermented loins were replicated independently twice. For each replicate, thirty-two fermented loin samples were produced (eight per treatment). At the end of production, proximate chemical composition, pH, water activity, thiobarbituric acid reactive substances (TBARS), color parameters, N-nitrosamines content and nitrate and nitrite residues were determined, whereas samples at one, four and six months of storage were tested for pH, water activity, TBARS, color parameters, N-nitrosamines content and nitrate and nitrite residues. The cross-sectional appearance of fermented loins after the production process and after six months of storage are presented in Figure 1.

Table 1. Formulations of fermented loin treatment, production and storage conditions.

	FL0	FL50	FL100	FL150
Sodium nitrate (mg kg^{-1})	0	50	100	150
fermentation chambers under controlled humidity (RH) and temperature (T)				
Production conditions	Stage 1 T 20–22 °C RH 55–63% 3 days	Stage 2 T 14–16 °C RH 68–75% 3 days	Stage 3 T 13 °C RH 76% 24 days	
Storage conditions	vacuum packed, 4 °C, 6 months			

FL0 (sample without sodium nitrate addition); FL50 (sample with reduced amount of sodium nitrate to 50 mg kg^{-1}); FL100 (sample with reduced amount of sodium nitrate to 100 mg kg^{-1}); FL150 (sample with traditional amount of sodium nitrate 150 mg kg^{-1}).



Figure 1. Cross-sectional appearance of fermented loins (a) after the production process, (b) after six months of storage. FL0 (sample without sodium nitrate addition); FL50 (sample with reduced amount of sodium nitrate to 50 mg kg⁻¹); FL100 (sample with reduced amount of sodium nitrate to 100 mg kg⁻¹); FL150 (sample with traditional amount of sodium nitrate 150 mg kg⁻¹).

2.2. Proximate Chemical Composition, Physicochemical Properties

The moisture, protein, collagen, and fat content in fermented loins were analyzed on comminuted samples (approximately 200 g sample) using Food Scan Lab 78,810 (Foss Tecator Co., Ltd., Hillerod, Denmark). The pH of fermented loins was determined on homogenates prepared by homogenizing 10 g of the ground sample with 50 cm³ of distilled water. For the measurements, a digital temperature-compensated pH meter (CPC-501, Elmetron, Zabrze, Poland) with a pH electrode (ERH-111, Hydromet, Gliwice, Poland) was used. Water activity (a_w) of the ground samples was measured at 20 °C using the LabMASTER-Aw system (Novasina AG, Lachen, Switzerland). Three measurements were made for each sample.

2.3. Analysis of Lipid Oxidation, Total Heme Pigments and Heme Iron Content

The content of secondary lipid oxidation products was used to assess the intensity of changes in the lipid fraction. Measuring the amount of thiobarbituric acid reactive substances (TBA) was performed according to the procedure described by Pikul et al. [23]. Absorbance was measured at 532 nm using a UV-visible spectrophotometer (Nicolet Evolution 300, Thermo Electron Corp., Waltham, MA, USA). The values were expressed as mg of malondialdehyde (MDA) per kilogram of sample. The total heme pigments and heme iron were determined following the analytical conditions described by Hornsey [24] with a slight modification described in a previous study [25]. The total pigments and heme iron concentration in the sample were calculated according to the procedure by Lee et al. [26] and expressed in mg kg⁻¹. Three measurements were made for each sample.

2.4. Color Measurements

A 10-mm-thick sample was cut from fermented loins to measure the color parameters (L*—lightness, a*—redness, b*—yellowness). An X-Rite 8200 colorimeter (X-Rite, Inc., Michigan, USA) calibrated using the black glasses and white tiles was used. The measurement was made on the cross-section immediately after cutting. The instrumental conditions were a 12-mm-diameter area aperture. The measurement was carried out in the range of 360 to 740 nm. The illuminant D65 and a 10° standard colorimetric observer was used as a source of light. The results were expressed in units of the CIE LAB [27] system.

Three measurements were made for each sample. The color difference (ΔE) during storage was calculated according to AMSA [28] using formula:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

2.5. N-Nitrosamines Determination

Volatile N-nitrosamines were analyzed according to the method of Drabik Markiewicz et al. [29] and DeMey et al. [7]. For the detection and quantification of selected N-nitrosamines including NDBA (N-nitrosodibutylamine), NDMA (N-nitrosodimethylamine), NDEA (N-nitrosodiethylamine), NDBA (N-nitrosodibutylamine), NMOR (N-nitrosomorpholine), NPIP (N-nitrosopiperidine), NPYR (N-nitrosopyrrolidine), a gas chromatograph coupled to a thermal energy analyzer (z. B, GC 7890B/Thermal Energy Analyser z. B, Tea 810 von der Fa, Ellutia, Cambridgeshire, UK) was used. Argon was used as a carrier gas (25 mL min⁻¹). The injection port of the GC went in a ramp from 155 to 255 °C and the oven temperature was increased from 60 °C to 250 °C at 5 °C min⁻¹.

2.6. Nitrate and Nitrite Residues

A total of 10 g of fermented loin sample was homogenized in water and clarified with acetonitrile before taking the measurement by ion-exchange chromatography (IC) and ultraviolet (UV) detection at 205 nm. The nitrate and nitrite residues were measured using FIAstarTM 5000 Analyzer (Foos, Denmark) equipment. The content of nitrate and nitrite residues was given as mg kg⁻¹ NaNO₂ and NaNO₃, respectively [30].

2.7. Statistical Analysis

The data reported were analyzed by two-way analysis of variance (ANOVA) using Statistica v. 13.3 software (Del. Inc., Round Rock, TX, USA). Effects between categorical factors (months and treatments) were analyzed. The significance of the differences between treatments at the same storage time and the same treatments at different storage times was determined using the Tukey's test. Differences at $p \leq 0.05$ were considered as significant. The results were expressed as the mean \pm standard deviation.

3. Results

3.1. Proximate Chemical Composition and Physicochemical Properties of Raw Material

The basic technological parameters (pH, water activity and color parameters) and the proximate chemical composition of the raw materials are shown in Table 2. The values of physicochemical properties (i.e., pH = 5.28, a_w = 0.989) were typical for good-quality fresh pork. The raw materials have a total pigment content of 72.31 mg kg⁻¹, which is also manifested by a relatively high redness value (a^* = 6.21). Likewise, the chemical composition is typical of the raw pork used. This is also confirmed by the content of heme pigments. The low value of TBARS (0.41 mg kg⁻¹) indicates a low content of substances reacting with thiobarbituric acid, the main representative of which is malondialdehyde, a secondary product of fat oxidation.

Table 2. Proximate chemical composition and basic technological parameters characterizing the raw materials (mean \pm standard deviation).

		Raw Material (Meat)
Proximate chemical composition [%]	Fat	3.92 \pm 0.83
	Protein	21.30 \pm 0.99
	Moisture	73.45 \pm 1.81
	Collagen	1.08 \pm 0.64
Basic technological parameters	pH	5.28 \pm 0.02
	a _w	0.989 \pm 0.003
	L*	63.71 \pm 4.64
	a*	6.21 \pm 1.29
	b*	15.60 \pm 1.30
Total pigment content [mg kg ⁻¹]		72.31 \pm 4.27
Heme iron content [mg kg ⁻¹]		6.30 \pm 0.40
TBARS [mg kg ⁻¹]		0.41 \pm 0.03

a_w—water activity, L*—lightness, a*—redness, b*—yellowness.

3.2. Chemical Composition and Physicochemical Properties of Fermented Loins

The moisture, protein, collagen and fat content of the fermented loins is shown in Table 3. As expected, the proximate chemical composition of samples were not affected by the level of nitrate. Meat products were characterized by a high protein content ranging from 26.72 to 28.91%. The salt concentration in four formulations of fermented loins was similar due to the same amount added during production and a similar degree of drying of the products during processing. The moisture content was in the range of 65.15 to 66.43%.

Table 3. Proximate chemical composition of fermented loins (mean \pm standard deviation).

Component [%]	FL0	FL50	FL100	FL150
Fat	4.21 \pm 0.24	4.39 \pm 0.48	3.86 \pm 0.42	4.80 \pm 0.53
Protein	27.82 \pm 0.71	28.57 \pm 0.73	28.91 \pm 0.73	26.72 \pm 0.68
Moisture	66.29 \pm 3.41	65.15 \pm 3.26	65.51 \pm 3.28	66.43 \pm 3.32
Collagen	0.78 \pm 0.19	0.72 \pm 0.15	0.76 \pm 0.13	1.05 \pm 0.21
Salt	1.73 \pm 0.13	1.73 \pm 0.11	1.81 \pm 0.11	1.73 \pm 0.08

No significant differences between samples were found. FL0 (sample without sodium nitrate addition); FL50 (sample with reduced amount of sodium nitrate to 50 mg kg⁻¹); FL100 (sample with reduced amount of sodium nitrate to 100 mg kg⁻¹); FL150 (sample with traditional amount of sodium nitrate 150 mg kg⁻¹).

Table 4 shows the pH and water activity values of fermented loins with varying levels of nitrate. Statistical analysis showed that the process factors such as treatments and storage time influenced ($p \leq 0.05$) the pH and water activity. At the beginning of the storage (month zero), lower pH values were detected for the samples containing nitrate at the level 100 and 150 mg kg⁻¹ compared to the samples FL0 and FL50. No statistically significant changes in pH were observed for the FL0 and FL50 samples with a storage time. Samples of fermented loin with higher levels of added nitrate (FL100, FL150) in four months of storage were characterized by a significantly higher pH compared to the previous storage periods. Water activity did not differ statistically significantly between the formulations. The a_w values ranged from 0.917 to 0.940 during the six-month storage period. With increased storage time, a decrease in water activity was observed for all samples. The lowest values were found in the sixth month of storage (0.917–0.922).

Table 4. pH and water activity of fermented loins during storage (mean \pm standard deviation).

	Storage Time [Month]	FL0	FL50	FL100	FL150
a_w	0	0.936 \pm 0.003 ^{a,C}	0.940 \pm 0.002 ^{a,B}	0.935 \pm 0.006 ^{a,B}	0.936 \pm 0.003 ^{a,B}
	1	0.926 \pm 0.004 ^{a,B}	0.928 \pm 0.005 ^{a,A}	0.935 \pm 0.006 ^{a,B}	0.923 \pm 0.010 ^{a,A}
	4	0.926 \pm 0.006 ^{a,B}	0.928 \pm 0.005 ^{a,A}	0.934 \pm 0.005 ^{a,B}	0.927 \pm 0.002 ^{a,A}
	6	0.917 \pm 0.003 ^{a,A}	0.922 \pm 0.003 ^{a,A}	0.920 \pm 0.002 ^{a,A}	0.921 \pm 0.003 ^{a,A}
pH	0	5.86 \pm 0.05 ^{a,A}	5.84 \pm 0.06 ^{a,A}	5.76 \pm 0.07 ^{b,A}	5.74 \pm 0.05 ^{b,A}
	1	5.95 \pm 0.18 ^{a,A}	5.92 \pm 0.11 ^{a,A}	5.93 \pm 0.11 ^{a,A}	5.72 \pm 0.15 ^{a,A}
	4	6.14 \pm 0.15 ^{a,A}	6.03 \pm 0.13 ^{a,A}	6.08 \pm 0.12 ^{a,B}	6.00 \pm 0.10 ^{a,B}
	6	5.96 \pm 0.08 ^{a,A}	5.87 \pm 0.08 ^{a,A}	5.86 \pm 0.07 ^{a,A}	5.82 \pm 0.06 ^{a,A}

a_w —water activity. FL0 (sample without sodium nitrate addition); FL50 (sample with reduced amount of sodium nitrate to 50 mg kg⁻¹); FL100 (sample with reduced amount of sodium nitrate to 100 mg kg⁻¹); FL150 (sample with traditional amount of sodium nitrate 150 mg kg⁻¹). ^{A-C} means followed by the common letters within the storage time do not differ significantly ($p \geq 0.05$); ^{a-b} means followed by the common letters within the formulations do not differ significantly ($p \geq 0.05$).

Regarding lipid oxidation, TBARS values were significantly higher in fermented loins without nitrate (FL0) and the lowest level of nitrate addition (FL50) compared to samples FL100 and FL150 at the end of processing (month zero) and after one month of storage (Table 5). An increase in TBARS in the case of all formulations was observed in four months of storage. Then, it significantly decreased after six months of storage to the lowest value in the entire storage period (1.47 to 1.64 mg kg⁻¹). Both in month four and six, no statistically significant ($p \geq 0.05$) differences in TBARS values were found between the formulations.

Table 5. TBARS, total pigment content and heme iron content in fermented loins during storage (mean \pm standard deviation).

	Storage Time [Month]	FL0	FL50	FL100	FL150
TBARS [mg kg ⁻¹]	0	1.63 \pm 0.13 ^{b,A,B}	1.40 \pm 0.12 ^{b,A}	1.20 \pm 0.11 ^{a,B}	1.04 \pm 0.13 ^{a,A}
	1	1.80 \pm 0.46 ^{b,B,C}	1.88 \pm 0.18 ^{b,B}	1.16 \pm 0.10 ^{a,A}	1.13 \pm 0.06 ^{a,A}
	4	2.27 \pm 0.66 ^{a,C}	2.60 \pm 0.46 ^{a,C}	2.22 \pm 0.86 ^{a,C}	2.26 \pm 0.52 ^{a,C}
	6	1.51 \pm 0.05 ^{a,A}	1.47 \pm 0.05 ^{a,A}	1.60 \pm 0.06 ^{a,B}	1.64 \pm 0.07 ^{a,B}
Total pigment content [mg kg ⁻¹]	0	109.7 \pm 18.4 ^{a,B}	101.8 \pm 6.4 ^{a,B}	96.6 \pm 5.9 ^{a,B}	91.7 \pm 10.9 ^{a,B}
	1	65.4 \pm 9.1 ^{a,A}	60.6 \pm 2.8 ^{a,A}	65.7 \pm 8.2 ^{a,A}	66.5 \pm 8.1 ^{a,A}
	4	72.1 \pm 11.9 ^{a,A}	69.6 \pm 6.2 ^{a,A}	63.6 \pm 3.5 ^{a,A}	65.0 \pm 6.4 ^{a,A}
	6	68.3 \pm 12.4 ^{a,A}	71.2 \pm 4.1 ^{a,A}	69.9 \pm 3.7 ^{a,A}	68.1 \pm 5.9 ^{a,A}
Heme iron content [mg kg ⁻¹]	0	9.7 \pm 2.5 ^{a,B}	9.0 \pm 0.6 ^{a,B}	8.5 \pm 0.5 ^{a,B}	7.4 \pm 1.0 ^{a,B}
	1	4.9 \pm 0.8 ^{a,A}	5.3 \pm 0.2 ^{a,A}	5.8 \pm 1.6 ^{a,A}	4.2 \pm 1.7 ^{a,A}
	4	7.1 \pm 1.7 ^{a,A}	6.1 \pm 0.5 ^{a,A}	5.6 \pm 0.3 ^{a,A}	5.4 \pm 0.2 ^{a,A}
	6	6.0 \pm 0.2 ^{a,A}	6.3 \pm 0.4 ^{a,A}	6.2 \pm 0.1 ^{a,A}	4.7 \pm 0.5 ^{a,A}

TBARS—thiobarbituric acid reactive substances. FL0 (sample without sodium nitrate addition); FL50 (sample with reduced amount of sodium nitrate to 50 mg kg⁻¹); FL100 (sample with reduced amount of sodium nitrate to 100 mg kg⁻¹); FL150 (sample with traditional amount of sodium nitrate 150 mg kg⁻¹). ^{A-C} means followed by the common letters within the storage time do not differ significantly ($p \geq 0.05$); ^{a-b} means followed by the common letters within the formulations do not differ significantly ($p \geq 0.05$).

The elimination or reduction of nitrate did not lead to statistically significant changes in total pigment content as well as heme iron content in fermented loins at zero, one, four and six months of storage (Table 5). However, statistically significant differences were observed considering the storage time. At the end of production, all the samples were characterized by a significantly higher total pigment and iron content compared to the subsequent research periods. After one month, a significant reduction in the value of these components was observed. They remained unchanged for the following months included in the experiment.

Figure 1 presented the cross-sectional appearance of fermented loins after the production process and after six months of storage. The cross-sectional appearance indicates greater differences in the color of the products after the end of ripening compared to those stored for six months.

The L^* , a^* and b^* values of fermented loin samples during storage are shown in Table 6. It was seen that the level of sodium nitrate addition had a statistically significant effect on lightness (L^*) and redness (a^*) values. The reduction of nitrate decreased the value of the L^* color parameter at the end of production (month zero). In the following months of storage, the differences between the formulations were in many cases statistically insignificant ($p \geq 0.05$). With prolonged storage time, especially between the first and fourth month, the lightness value decreased for the samples without the addition of nitrate (FL0) and with its lowest level (FL50). The greatest effect of the level of nitrate addition and the storage time was noted for the a^* color parameter determining redness. The lowest redness was recorded in the samples without nitrate (FL0) at the end of production (month zero), while the highest values were noted in the FL50 sample at month one. The differences in the redness value were not statistically significant between the FL100 and FL150 samples. The color differences (ΔE) between fermented loin samples with various amounts of sodium nitrate showed that the greatest color changes took place during production (change calculated from the color of the meat and product at the end of production). During storage, the color changes for the samples ranged from 2.47 to 9.75. The greatest color changes were shown by the FL150 sample.

Table 6. CIE L^* a^* b^* color parameters of fermented loins during storage (mean \pm standard deviation).

		Storage Time [Month]			
		0	1	4	6
L^*	FL0	51.51 \pm 4.07 ^{a,A}	51.42 \pm 1.62 ^{a,A}	43.86 \pm 5.53 ^{a,B}	40.58 \pm 1.22 ^{a,B}
	FL50	47.14 \pm 2.40 ^{a,A}	43.89 \pm 4.35 ^{b,B}	40.66 \pm 3.19 ^{a,B,C}	37.62 \pm 1.39 ^{a,C}
	FL100	45.84 \pm 2.75 ^{b,A}	41.60 \pm 4.58 ^{b,A}	43.99 \pm 2.64 ^{a,A}	40.74 \pm 1.16 ^{a,A}
	FL150	42.48 \pm 2.56 ^{b,A}	51.14 \pm 4.75 ^{a,B}	44.38 \pm 2.90 ^{a,A}	41.31 \pm 0.93 ^{a,A}
a^*	FL0	2.20 \pm 0.93 ^{a,A}	4.19 \pm 0.59 ^{a,B}	4.89 \pm 1.18 ^{a,B}	4.72 \pm 0.68 ^{a,B}
	FL50	6.30 \pm 0.57 ^{b,A}	8.25 \pm 0.98 ^{b,B}	7.43 \pm 0.47 ^{b,B}	8.05 \pm 0.83 ^{b,B}
	FL100	6.26 \pm 0.90 ^{b,A}	5.52 \pm 1.71 ^{a,A}	5.32 \pm 1.15 ^{a,A}	7.19 \pm 0.56 ^{b,B}
	FL150	4.94 \pm 0.76 ^{b,A}	5.80 \pm 1.06 ^{a,A}	6.96 \pm 1.28 ^{b,a,B}	5.85 \pm 1.10 ^{b,A}
b^*	FL0	8.30 \pm 1.10 ^{a,A}	9.89 \pm 0.65 ^{a,A}	8.61 \pm 1.99 ^{a,A}	7.30 \pm 0.57 ^{a,A}
	FL50	7.22 \pm 0.87 ^{a,A}	8.43 \pm 1.27 ^{a,A}	8.09 \pm 0.85 ^{a,A}	7.93 \pm 0.72 ^{a,A}
	FL100	7.32 \pm 0.69 ^{a,A}	6.34 \pm 0.77 ^{a,A}	7.10 \pm 1.37 ^{a,A}	8.87 \pm 0.93 ^{a,B}
	FL150	5.83 \pm 1.08 ^{a,A}	9.24 \pm 1.46 ^{a,B}	8.40 \pm 0.89 ^{a,B}	7.66 \pm 1.07 ^{a,B}
ΔE	FL0	14.77 \pm 1.69 ^{a,C}	2.54 \pm 0.44 ^{a,A}	7.68 \pm 1.04 ^{b,B}	3.53 \pm 0.98 ^{a,A}
	FL50	18.31 \pm 1.34 ^{b,B}	3.97 \pm 1.01 ^{a,A}	3.35 \pm 0.23 ^{a,A}	3.11 \pm 1.00 ^{a,A}
	FL100	16.69 \pm 1.52 ^{a,b,B}	4.41 \pm 0.74 ^{a,A}	2.47 \pm 0.75 ^{a,A}	4.67 \pm 1.04 ^{a,A}
	FL150	23.28 \pm 2.04 ^{c,C}	9.75 \pm 0.94 ^{b,B}	6.85 \pm 1.09 ^{b,B}	3.34 \pm 0.84 ^{a,A}

L^* —lightness, a^* —redness, b^* —yellowness, ΔE —color difference. FL0 (sample without sodium nitrate addition); FL50 (sample with reduced amount of sodium nitrate to 50 mg kg⁻¹); FL100 (sample with reduced amount of sodium nitrate to 100 mg kg⁻¹); FL150 (sample with traditional amount of sodium nitrate 150 mg kg⁻¹). ^{A-C} means followed by the common letters within the storage time do not differ significantly ($p \geq 0.05$); ^{a-c} means followed by the common letters within the formulations do not differ significantly ($p \geq 0.05$).

The analysis of the formulations of fermented loins with different nitrate level additions at the end of processing revealed residual sodium nitrite levels of below 10 mg kg⁻¹ (Table 7). A higher amount of nitrate was noted ranging from <10 mg kg⁻¹ for the sample

with nitrate addition to 19.0 mg kg⁻¹ for the sample with the highest nitrate addition. With increasing levels of nitrate addition, higher nitrate residues were observed in fermented meat products. In all the tested samples, the level of nitrosamines (NDBA, NDEA, NDMA, NDPA, NMOR, NPIP, N-Nitrosopyrrolidin) was <5 µg kg⁻¹ at the end of processing, which proves their chemical safety.

Table 7. Nitrosamine, nitrite and nitrate residues in fermented loins after production.

Parameter	FL0	FL50	FL100	FL150
N-Nitrosodibutylamin (NDBA) [µg kg ⁻¹]	<5	<5	<5	<5
N-Nitrosodiethylamin (NDEA) [µg kg ⁻¹]	<5	<5	<5	<5
N-Nitrosodimethylamin (NDMA) [µg kg ⁻¹]	<5	<5	<5	<5
N-Nitrosodipropylamin (NDPA) [µg kg ⁻¹]	<5	<5	<5	<5
N-Nitrosomorpholin (NMOR) [µg kg ⁻¹]	<5	<5	<5	<5
N-Nitrosopiperidin (NPIP) [µg kg ⁻¹]	<5	<5	<5	<5
N-Nitrosopyrrolidin [µg kg ⁻¹]	<5	<5	<5	<5
NaNO ₂ [mg kg ⁻¹]	<10	<10	<10	<10
NaNO ₃ [mg kg ⁻¹]	<10	11.0 ± 2.0	17.0 ± 2.0	19.0 ± 1.0

FL0 (sample without sodium nitrate addition); FL50 (sample with reduced amount of sodium nitrate to 50 mg kg⁻¹); FL100 (sample with reduced amount of sodium nitrate to 100 mg kg⁻¹); FL150 (sample with traditional amount of sodium nitrate 150 mg kg⁻¹).

4. Discussion

The current study assessed the impact of the level of nitrate addition on the quality of traditionally produced fermented loins. It has been shown that changes in the basic meat components during production changed the chemical composition of the products as compared to the properties of the raw material. The values of the physicochemical properties of raw material used for fermented loin production were typical for good-quality fresh pork and similar to those obtained by Kęska and Stadnik [31]. Water loss during drying, by almost 10% compared to the moisture content in raw material, resulted in a higher concentration of nutrients, especially proteins. The fermented loins had a relatively high average protein content in the range of 26.72 to 28.91%. The significant loss of water during production implied also that the loins had a high total pigment content, over 20 mg kg⁻¹ more compared to the raw material at the end of production. The reduction of the water content and the biochemical changes taking place during fermentation and drying resulted in the reduction of the water activity to the value 0.917 to 0.940 depending of the storage time. The observed value of water activity indicated unfavorable conditions preventing the growth of the most popular bacteria in meat products such as *Listeria monocytogenes* and *Clostridium botulinum* [32]. With increased storage time, a decrease in water activity was observed for all fermented loins which may point to the production of low molecular weight nitrogen compounds from the decomposition of meat protein during storage by meat and microbial enzymes [33]. The average water activity in fermented loins with different levels of nitrate was similar to that found by Stadnik et al. [34] for dry-cured loins. Unfortunately, no decrease in pH was achieved during the production in relation to the raw material, which indicates insufficient lactic acid bacteria growth during production. The increase of pH value during processing may result from many factors including proteolytic changes. According to Berardo et al. [35], as a result of proteolysis, which causes, among others, protein breakdown, decarboxylation and deamination, substances such as ammonia and amines can be released. In this context, the progressive increase in the content of these compounds reduces the acidity of fermented meat products.

A very important aspect of the quality of fermented meat products is the oxidative stability of the lipids they contain. At the beginning of the storage period, the TBARS values were significantly higher in fermented loins without nitrate and with the lowest

level of nitrate addition compared to samples with the nitrate addition in the amount of 100 and 150 mg kg⁻¹. This indicates the protective role of nitrate as an antioxidant component in meat products. Various mechanisms of the antioxidant effect of nitrate are described in the literature, including binding of heme and the prevention of the release of the catalytic non-heme iron, the ability to bind heme and non-heme iron and inhibit catalysis, and the stabilization of lipids against oxidation [5]. However, at the end of the storage period, meat products containing various amounts of nitrate presented the same amount of TBARS similar to the findings by Ferysiuk and Wójciak [36]. Eskandari et al. [37] noted that the addition of 40 and 120 mg kg⁻¹ of sodium(III) nitrite to frankfurters contributed to the effectively lowered amount of malondialdehyde during eight weeks of chilled storage. Our previous study, in which we assessed the effect of a reduced nitrate addition in the production of heat-treated meat products [19], showed that the reduction of the nitrate addition by 50 mg kg⁻¹ in relation to the traditionally used level did not significantly change the amount of secondary lipid oxidation products measured with the TBARS.

Apart from lipids, myoglobin can also undergo various processes resulting in changes in the quality characteristics of fermented meat products, including mainly the color and nutritional value in the context of heme iron content. In the current study, the reduction of nitrate did not lead to significant changes in total pigment content as well as heme iron content in fermented loins during six months of chilled storage. However, considering the storage time, at the end of production, all the samples were characterized by a significantly higher total pigment and iron content compared to the subsequent research periods. After one month, a significant reduction in the value of these components was observed. Saputro et al. [38] showed that the breakdown of heme pigments during storage might have led to the lower lightness value. This phenomenon has not been confirmed in the present study.

The color parameters L* and a* observed in the present work in the fermented loins depended on the level of sodium nitrate addition, especially at the end of production. The reduction of nitrate decreased the value of the L* color parameter and redness (a* color parameter) which is associated with the nitrosation of the myoglobin to nitrosomyoglobin. This form of myoglobin results from the interaction between muscle-based myoglobin and nitric oxide, which is derived from nitrate [2]. During fermentation, nitric oxide is formed in numerous chemical reactions among which reaction with reductants (present in the meat or added) are of practical importance. Numerous nitrosation and nitrosylation reactions take place in the meat matrix, among which the formation of nitrosomyoglobin is of major importance [39]. In the present study, fermented loins without the addition of nitrate were characterized by the lowest a* color parameter. However, the redness values obtained in the study were significantly higher compared to those obtained with the fermented loin without the addition of nitrates [34]. In contrast, the results presented by Karwowska et al. [19] showed no effect from the inclusion of different amounts of sodium nitrite on the lightness (L*) and redness (a*) values of cooked meat products. Interestingly, in Parma ham, which does not contain added nitrates, a stable red color compound is formed, in which iron is substituted by zinc in the myoglobin molecule. In the study by Parolari et al. [40], nitrite-free dried hams developed a stable color through the endogenous synthesis of red Zn-protoporphyrin IX, according to a natural, enzyme-dependent process conducted at a warm maturation temperature. Wakamatsu et al. [41] documented the ability of the muscle ferrochelatase to catalyze the iron displacement from the heme moiety, a key step prior to the eventual insertion of zinc, leading to Zn protoporphyrin IX. The redness of fermented loins with reduced or no nitrate added observed after storage can be also the result of this phenomenon.

Fermented loins with different nitrate level additions revealed residual sodium nitrite levels below 10 mg kg⁻¹. At the same time, the samples were characterized by a higher nitrate residue, the highest amount (19.0 mg kg⁻¹) for the sample with the addition of 150 mg kg⁻¹ of nitrates during production. In the research by Iacumin et al. [42], 4 and 22 mg kg⁻¹ of nitrite and nitrate, respectively, were noted in dry-cured ham of 14 to

19 months, although the hams were not cured during production. This could indicate the presence of nitrates in the raw material, due to illegal practices of the producers or contamination of the feed. Ianmarino and Di Taranto [43] indicated a natural endogenous formation of nitrate with a maximum of 30 mg kg^{-1} in beef and pork meats due to feeding regimes. Similarly, Iacumin et al. [42] reported that the low levels of nitrites and nitrates in the fresh meat used in the meat industry may derived from the nitrogen metabolism of the animal. As reported of Sindelar and Milkowski [44], a residual nitrite level of 10 to 15 mg kg^{-1} is useful as and used for the regeneration of cured meat color as it prevents the reduction of the red color over the storage period.

The presence of nitrate and nitrite residues in meat products is correlated with the formation of N-nitrosamines; it is well-known that a low content of residual nitrates/nitrites inhibits the production of nitrosamines. In the current study, the level of nitrosamines in all samples regardless of the level of nitrate added was very low ($< 5 \text{ } \mu\text{g kg}^{-1}$). This proves the chemical safety of experimental meat products. Aschebrook-Kilfoy et al. [45] described the connection between the intake of nitrite of animal origin and thyroid cancer risk. They explained this by the specificity of meat as a source of precursors (amines and amides) for N-nitroso compounds that can cause thyroid cancer. According to several studies [46–48], it is possible that nitrates/nitrites of animal sources (rich in amines and amides and heme iron) could increase the risk of cancer by increasing the production of endogenous N-nitroso compounds. Hem is described in the literature as a nitrosating agent; nitrosyl heme contributes significantly to the endogenous production of N-nitroso compounds [49,50].

5. Conclusions

The negative health effects of nitrates and nitrites are commonly described in the literature. In this context, the elimination or significant reduction of nitrate content in view of obtaining a product with a “clean label” by replacing curing with salting increases the nutritional value of meat products and consumer confidence. The results obtained in the current study show that the reduction of nitrite to the level of 50 mg kg^{-1} allows to obtain fermented loins with physicochemical characteristics similar to the product with the traditional use of nitrite (150 mg kg^{-1}). Moreover, fermented loins with 50 mg kg^{-1} addition of nitrite are characterized by similar TBARS values during six months of storage as well as heme iron content. The reduction of the nitrate addition to the level of 50 mg kg^{-1} results in almost two-fold lower nitrate residues. This reduces the risk of nitrosation in the gastrointestinal tract with the formation of nitro compounds. This allows the production of high-quality meat products with higher nutritional value and that are better perceived by consumers. However, further research in this regard is needed to see if such a low residual nitrate level is sufficient to inhibit *C. botulinum* spore outgrowth and the growth of other pathogens typical of meat products.

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References

1. Flores, M.; Toldrá, F. Chemistry, safety, and regulatory considerations in the use of nitrite and nitrate from natural origin in meat products—Invited review. *Meat Sci.* **2021**, *171*, 108272. [CrossRef]
2. Hammes, P.W. Metabolism of nitrate in fermented meats: The characteristic feature of a specific group of fermented foods. *Food Microbiol.* **2012**, *29*, 151–156. [CrossRef]
3. Honikel, K.O. Curing agents. In *Encyclopedia of Meat Sciences*; Jensen, W.K., Devine, C., Dikeman, M., Eds.; Elsevier Ltd.: Oxford, UK, 2004; pp. 195–201.
4. Flores, M. Understanding the implications of current health trends on the aroma of wet and dry cured meat products. *Meat Sci.* **2018**, *144*, 53–61. [CrossRef]
5. Weiss, J.; Gibis, M.; Schuh, V.; Salminen, H. Advances in ingredient and processing systems for meat and meat products. *Meat Sci.* **2010**, *86*, 196–213. [CrossRef] [PubMed]
6. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Lyon, France. 2018. Available online: <https://monographs.iarc.fr/wp-content/uploads/2018/06/mono114.pdf> (accessed on 5 January 2021).
7. De Mey, E.; De Klerck, K.; De Maere, H.; Dewulf, L.; Derdelinckx, G.; Peeters, M.-C.; Fraeye, I.; Heyden, Y.V.; Paelinck, H. The occurrence of N-nitrosamines, residual nitrite and biogenic amines in commercial dry fermented sausages and evaluation of their occasional relation. *Meat Sci.* **2014**, *96*, 821–828. [CrossRef]
8. Pegg, R. *Handbook of Fermented Meat and Poultry*, 2nd ed.; Wiley-Blackwell: Chichester, UK, 2015; pp. 19–30.
9. Sindelar, J.J.; Milkowski, A.L. Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide* **2012**, *26*, 259–266. [CrossRef]
10. Ma, L.; Hu, L.; Feng, X.; Wang, S. Nitrate and nitrite in health and disease. *Aging Dis.* **2018**, *9*, 938–945. [CrossRef]
11. D’Ischia, M.; Napolitano, A.; Manini, P.; Panzella, L. Secondary targets of nitrite-derived reactive nitrogen species: Nitrosation/nitration pathways, antioxidant defense mechanisms and toxicological implications. *Chem. Res. Toxicol.* **2011**, *24*, 2071–2092. [CrossRef]
12. Bryan, N.S. Dietary nitrite: From menace to marvel. *Funct. Foods Health Dis.* **2016**, *6*, 691–701. [CrossRef]
13. Raubenheimer, K.; Bondonno, C.; Blekkenhorst, L.; Wagner, K.-H.; Peake, J.-M.; Neubauer, O. Effect of dietary nitrate on inflammation and immune function and implications for cardiovascular health. *Nutr. Rev.* **2019**, *77*, 584–599. [CrossRef] [PubMed]
14. Jakobsen, J.; Hansen, A. Geographies of meatification: An emerging Asian meat complex. *Globalizations* **2020**, *17*, 93–109. [CrossRef]
15. Commission Regulation (EU). No. 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No. 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. *Off. J. Eur. Union* **2011**, *295*, 1–177.
16. Munekata, P.E.S.; Pateiro, M.; Domínguez, R.; Santos, E.M.; Lorenzo, J.M. Cruciferous vegetables as sources of nitrate in meat products. *Curr. Opin. Food Sci.* **2021**, *38*, 1–7. [CrossRef]
17. Rivera, N.; Bunning, M.; Martin, J. Uncured-labeled meat products produced using plant-derived nitrates and nitrites: Chemistry, safety, and regulatory considerations. *J. Agric. Food Chem.* **2019**, *67*, 8074–8084. [CrossRef]
18. Wójciak, K.M.; Stasiak, D.; Kęska, P. The influence of different levels of sodium nitrite on the safety, oxidative stability, and color of minced roasted beef. *Sustainability* **2019**, *11*, 3795. [CrossRef]
19. Karwowska, M.; Kononiuk, A.; Wójciak, K. Impact of sodium nitrite reduction on lipid oxidation and antioxidant properties of cooked meat products. *Antioxidants* **2020**, *9*, 9. [CrossRef]
20. UNI. *Foodstuffs—Determination of Nitrate and/or Nitrite Content—Part 4: Ion-Exchange Chromatographic (IC) Method for the Determination of Nitrate and Nitrite Content of Meat Products*; UNI EN 12014-4; UNI: Milano, Italy, 2005.
21. Kononiuk, A.D.; Karwowska, M. Comparison of selected parameters related to food safety of fallow deer and beef uncured fermented sausages with freeze-dried acid whey addition. *Meat Sci.* **2020**, *161*, 108015. [CrossRef] [PubMed]
22. Kononiuk, A.D.; Karwowska, M. Influence of freeze-dried acid whey addition on biogenic amines formation in a beef and deer dry fermented sausages without added nitrite. *Asian-Austral. J. Anim. Sci.* **2020**, *33*, 332–338. [CrossRef] [PubMed]
23. Pikul, J.; Leszczynski, D.E.; Kummerow, F.A. Evaluation of three modified TBA methods for measuring lipid oxidation in chicken meat. *J. Agric. Food Chem.* **1989**, *37*, 1309–1313. [CrossRef]
24. Hornsey, H.C. The colour of cooked cured pork. I.—Estimation of the Nitric oxide-Haem Pigments. *J. Sci. Food Agric.* **1959**, *7*, 534–540. [CrossRef]
25. Karwowska, M.; Dolatowski, Z.J. Comparison of lipid and protein oxidation, total iron content and fatty acid profile of conventional and organic pork. *Int. J. Food Sci. Technol.* **2013**, *48*, 2200–2206. [CrossRef]
26. Lee, B.J.; Hendricks, D.G.; Cornforth, D.P. A comparison of carnosine and ascorbic acid on color and lipid stability in a ground beef patties model system. *Meat Sci.* **1999**, *51*, 245–253.
27. *Commission Internationale de l’Eclairage: Recommendations on Uniform Colour Spaces, Colour Difference Equations, Psychometric Color Terms*; Supplement No. 2; Bureal Central de la CIE: Paris, France, 1978.
28. AMSA. *Meat Color Measurements Guidelines*; American Meat Science Association: Savoy, IL, USA, 2012.
29. Drabik-Markiewicz, G.; Dejaegher, B.; De Mey, E.; Impens, S.; Kowalska, T.; Paelinck, H.; Heyden, Y.V. Evaluation of the influence of proline, hydroxyproline or pyrrolidine in the presence of sodium nitrite on N-nitrosamine formation when heating cured meat. *Anal. Chim. Acta* **2010**, *657*, 123–130. [CrossRef] [PubMed]

30. Kononiuk, A.; Karwowska, M. Bioactive Compounds in Fermented Sausages Prepared from Beef and Fallow Deer Meat with Acid Whey Addition. *Molecules* **2020**, *25*, 2429. [[CrossRef](#)] [[PubMed](#)]
31. Keška, P.; Stadnik, J. Combined effect of sonication and acid whey on antioxidant and angiotensin-converting enzyme inhibitory activities of peptides obtained from dry-cured pork loin. *Appl. Sci.* **2020**, *10*, 4521. [[CrossRef](#)]
32. Fontana, A.J. Appendix D: Minimum water activity limits for growth of microorganisms. In *Water Activity in Foods: Fundamentals and Applications*; Blackwell Publishing: Oxford, UK, 2007; p. 405.
33. Marra, A.I.; Salgado, A.; Prieto, B.; Carballo, J. Biochemical characteristics of dry-cured lacón. *Food Chem.* **1999**, *67*, 33–37. [[CrossRef](#)]
34. Stadnik, J.; Stasiak, D.M.; Dolatowski, Z.J. Proteolysis in dry-cured loins manufactured with sonicated pork and inoculated with *Lactobacillus casei* LOCK 0900 probiotic strain. *Int. J. Food Sci. Technol.* **2014**, *49*, 2578–2584. [[CrossRef](#)]
35. Berardo, A.; Devreese, B.; De Maere, H.; Stavropoulou, D.A.; Van Royen, G.; Leroy, F.; De Smet, S. Actin proteolysis during ripening of dry fermented sausages at different pH values. *Food Chem.* **2017**, *221*, 1322–1332. [[CrossRef](#)] [[PubMed](#)]
36. Ferysiuk, K.; Wójciak, K. The Possibility of Reduction of Synthetic Preservative E 250 in Canned Pork. *Foods* **2020**, *9*, 1869. [[CrossRef](#)]
37. Eskandari, M.H.; Hosseinpour, S.; Mesbahi, G.; Shekarforoush, S. New composite nitrite-free and low-nitrite meat-curing systems using natural colorants. *Food Sci. Nutr.* **2013**, *1*, 392–401. [[CrossRef](#)]
38. Saputro, E.; Bintoro, V.P.; Pramono, Y.B. Color, pigment and residual nitrite of *dendeng sapi* naturally cured at various level of celery leaves and incubation temperatures. *JITAA* **2016**, *41*, 99–105.
39. Honikel, K.O. The use and control of nitrate and nitrite for the processing of meat products. *Meat Sci.* **2008**, *78*, 68–76. [[CrossRef](#)] [[PubMed](#)]
40. Parolari, G.; Aguzzoni, A.; Toscani, T. Effects of Processing Temperature on Color Properties of Dry-Cured Hams Made without Nitrite. *Foods* **2016**, *5*, 33. [[CrossRef](#)] [[PubMed](#)]
41. Wakamatsu, J.; Okui, J.; Ikeda, Y.; Nishimura, T.; Hattori, A. Establishment of a model experiment system to elucidate the mechanism by which Zn-protoporphyrin IX is formed in nitrite-free dry-cured ham. *Meat Sci.* **2004**, *68*, 313–317. [[CrossRef](#)]
42. Iacumin, L.; Cattaneo, P.; Zuccolo, C.; Galanetto, S.; Acquafredda, A.; Comi, G. Natural levels of nitrites and nitrates in San Daniele dry cured ham PDO, and in meat, salt and sugna used for its production. *Food Control.* **2019**, *100*, 257–261. [[CrossRef](#)]
43. Iammarino, M.; Di Taranto, A.; Cristino, M. Endogenous levels of nitrites and nitrates in wide consumption foodstuffs: Results of five years of official controls and monitoring. *Food Chem.* **2013**, *140*, 763–771. [[CrossRef](#)]
44. Sindelar, J.J.; Cordray, J.C.; Sebranek, J.G.; Love, J.A.; Ahn, D.U. Effects of varying levels of vegetable juice powder and incubation time on color, residual nitrate and nitrite, pigment, pH, and trained sensory attributes of ready-to-eat uncured ham. *J. Food Sci.* **2007**, *72*, 388–395. [[CrossRef](#)]
45. Aschebrook-Kilfoy, B.; Shu, X.O.; Gao, Y.T.; Ji, B.T.; Yang, G.; Li, H.L.; Ward, M.H. Thyroid cancer risk and dietary nitrate and nitrite intake in the Shanghai women’s health study. *Int. J. Cancer* **2013**, *132*, 897–904. [[CrossRef](#)]
46. Van Hecke, T.; Vossen, E.; Hemeryck, L.Y.; Bussche, J.V.; Vanhaecke, L.; De Smet, S. Increased oxidative and nitrosative reactions during digestion could contribute to the association between well-done red meat consumption and colorectal cancer. *Food Chem.* **2015**, *187*, 29–36. [[CrossRef](#)]
47. Bastide, N.M.; Chenni, F.; Audebert, M.; Santarelli, R.L.; Taché, S.; Naud, N.; Kuhnle, G.G. A central role for heme iron in colon carcinogenesis associated with red meat intake. *Cancer Res.* **2015**, *75*, 870–879. [[CrossRef](#)]
48. Zheng, J.; Stu, J.; Tang, H.; Hassan, M.M.; Daniel, C.R.; Li, D. Dietary N-nitroso compounds and risk of pancreatic cancer: Results from a large case-control study. *Carcinogenesis* **2018**, *40*, 254–262. [[CrossRef](#)]
49. Cross, A.J.; Harnly, J.M.; Ferrucci, L.M.; Risch, A.; Mayne, S.T.; Sinha, R. Developing a heme iron database for meats according to meat type, cooking method and doneness level. *Food Nutr. Sci.* **2012**, *3*, 905–913. [[CrossRef](#)]
50. Hodra, J.; Shah, A.; Zhang, L. Heme, an Essential Nutrient from Dietary Proteins, Critically Impacts Diverse Physiological and Pathological Processes. *Nutrients* **2014**, *6*, 1080–1102. [[CrossRef](#)] [[PubMed](#)]