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Comparative Studies on the Fatty Acid Profile and Volatile Compounds of Fallow Deer and Beef Fermented Sausages without Nitrite Produced with the Addition of Acid Whey

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Featured Application: It has been proven to be possible to eliminate nitrite from the production of fermented beef and fallow deer sausages without changing the amount of volatile compounds. However, the addition the freeze dried acid whey powder (0.7%) resulted the changes in the amount of some volatile components, in particular those derived from bacterial metabolism, which indicates the need for a sensory evaluation of products to show whether this does not affect the characteristics of its acceptability.

Abstract: This study aims to improve knowledge on fermented beef and fallow deer sausages and the effect of nitrite elimination and the addition of freeze dried acid whey on the fatty acid profile and volatile compounds. Three different formulations within each of the two product groups, made of beef and fallow deer meat, respectively, were prepared: control sample with sodium nitrite, sample without nitrite, and sample without nitrite and with the addition of freeze-dried acid whey powder (0.7%). After production, the sausages were subjected to analysis including proximate chemical composition, pH and water activity, Thiobarbituric Acid Reactive Substance (TBARS), fatty acid profile, and volatile compound determination. The fermented sausages were characterized by an average pH and water activity in the range of 5.23–5.79 and 0.910–0.918, respectively. Fallow deer sausages were characterized by a higher content of saturated and polyunsaturated fatty acids in comparison to beef sausages. The elimination of nitrite did not significantly affect the amount of volatile compounds in fermented sausages. However, the effect of the freeze-dried acid whey powder addition on the amount of some volatile compounds in uncured sausages was observed, in particular, that derived from bacterial metabolism.

Keywords: fermented sausage; beef; fallow deer; acid whey; fatty acid profile; volatile compounds

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1. Introduction

Fermented meat products are a traditional and high-quality food that is an important part of the European cultural heritage [1]. They are traditionally manufactured using nitrate and nitrite [2]. However, in recent years, their unique nutritional properties have been emphasized by eliminating or reducing synthetic additives in the direction of the "clean label" trend. Such products are considered to be healthy and much more preferred by modern consumer [3]. Nitrates and nitrites are controversial additives in meat processing due to their involvement in the formation of nitroso-compounds, such as carcinogenic N-nitrosoamines [4]. To obtain clean label products, natural raw materials of plant or animal

origin are used [5–8] whose aim is to impart similar properties to meat products compared to products with synthetic additives. However, the elimination of nitrates/nitrites carries the risk of reducing the shelf life of meat products as these compounds are added as preservatives due to their antimicrobial activity and antioxidants properties. The beneficial effect of nitrites/nitrates in meat products is also related to the positive effect of color enhancement and the development of the flavor typical of cured meat products [9–11]. Our previous studies showed the possibility of using freeze-dried acid whey as a nitrite substitute in fermented sausages [12–14]. The addition of acid whey in uncured dry fermented sausage improved its properties including the nutritional value by improving the CLA. It was indicated that the acid whey had a similar effect on the tested parameters as nitrate/nitrite in fermented sausages. The efforts of scientists and meat processors to reduce nitrites are particularly important in the case of red meat, which includes ruminant meat (e.g., beef and fallow deer). It is well known that a high consumption of red and processed meat (e.g., cured) may exert some toxic effects on humans, such as cancer risk, the risk of heart diseases, and different metabolic disorders (diabetes, weight gain) [15,16].

During the production of fermented meat products, as a result of the ripening process, complex chemical and biochemical changes in the main components of meat including proteins and lipids lead to the generation of flavor precursors such as peptides, free amino acids, and free fatty acids, which are transformed to volatile compounds [17–21]. Volatile compound composition might be due to seasoning, smoking or the reactions between lipids, proteins, and carbohydrates caused by microbial or endogenous enzymes [22]. Lipolysis and proteolysis are essential biochemical reactions for volatile compound development as they provide precursors, free amino acids, and fatty acids, which will be further degraded to produce aroma compounds [23–25]. Nitrates/nitrites play a significant role in the development of the unique cured meat flavor; however, the mechanism is not fully understood. When nitrites are bound to sulfur-containing amino acids of meat proteins, SH-residues with a specific aroma and flavor are formed and contribute to the unique flavor of cured meat. To the best of our knowledge, few studies have examined the effect of nitrate/nitrite reduction on the aroma development in meat products. The trend in volatile compound studies is to report the changes produced in the volatile profile due to different factors (e.g., salt reduction, fat content) and the identification of hundreds of volatile compounds [20,26]. The study by Hospital et al. [27] reported an effect on volatile compound formation in dry sausages with reduced nitrate/nitrite addition. The sausages with reduced curing agents contained the highest amounts of volatile compounds derived from carbohydrate fermentation and amino acid degradation reactions, probably produced by the increased growth of Gram-positive catalase-positive cocci and *Enterobacteriaceae*. To the best of our knowledge, there are no studies in the global literature on the effect of acid whey on the content of volatile compounds in fermented sausages made from ruminant meat without nitrite. Thus, the aim of this work was to improve our knowledge on fermented beef and fallow deer sausages and the effect of nitrite elimination and the addition of freeze dried acid whey (0.7%) on the fatty acid profile and volatile compounds.

2. Materials and Methods

2.1. Fermented Sausage Manufacturing

The experiment was replicated three times by producing three different batches on separate days. In each replicate, two types of sausages were prepared—beef and fallow deer, each consisting of three different formulations as presented in Table 1: C—control sample with sodium nitrite, S—sample with sea salt, SAW—sample with the addition of sea salt and freeze-dried acid whey (equivalent to 10% of liquid acid whey). The raw materials for the production of fermented sausages were fallow deer meat and tallow as well as beef and beef tallow. The fallow deer meat and tallow came from a certified as organic breeding farm (Przytoczno Farm, Przytoczno, Poland), where animals live in natural environmental conditions. Organic beef and beef fallow were obtained from a butcher (Wasąg, Biłgoraj, Poland). Minced meat and tallow in a ratio 9:1 were used for sausage preparation. Sea

salt (non-iodinated and without anti-caking agents) (CuroDiMare, Saline di Margherita di Savoia, Apulia, Italy) and glucose (Delecta, Warsaw, Poland) were purchased from local supermarkets (Lublin, Poland). Sodium nitrite (without anti-caking agents) was obtained from StanLab (Lublin, Poland). Organic acid whey was bought fresh from a certified diary product plant (R. Janowski, Ludwinów, Poland) and then it was lyophilized using a freeze-drier (Labconco Free-Zone, Labconco Corporation, Kansas City, MO, USA). Acid whey powder was stored at −50 °C until sausage production. Shortly before use, it was dissolved in saline for better spreading in food stuffs. Sausage variants were prepared by aging in fermentation chambers under controlled humidity (80 ± 5%) and temperature (16 ± 1 °C) conditions until 30% weight loss (about 20 days). After production, the samples were subjected to analysis including proximate chemical composition, pH and water activity (aw), lipid oxidation, fatty acid profile, and volatile compound determination.

Table 1. Formulation of beef and fallow deer sausage treatments.

Ingredients (%)	C	S	SAW
Glucose	0.6	0.6	0.6
Sea salt	2.786	2.8	2.8
Sodium nitrite	0.014	-	-
Acid whey powder	-	-	0.70
Water/Saline	5	5	5

C—Sample with curing mixture, S—sample with sea salt, SAW—sample with the addition of sea salt and freeze-dried acid whey (equivalent to 10% of liquid acid whey).

2.2. Proximate Chemical Composition, pH, and Water Activity (a_w) Determination

A Food Scan Lab 78810 (Foss Tecator Co., Ltd., Hillerod, Denmark) was used to determine the moisture, protein, collagen, and fat content. Approximately 200 g of a homogenized sausage sample (each) was distributed in the instrument's round sample dish and loaded into the instrument's sample chamber. The pH of sausage homogenates was measured with a digital temperature-compensated pH meter (CPC-501, Elmetron, Zabrze, Poland) with a pH electrode (ERH-111, Hydromet, Gliwice, Poland) calibrated with buffer solutions (pH 4.0, 7.0, 9.0). The water activity (a_w) of the ground sausage samples was measured using a water activity analyzer (Novasina AG, Lachen, Switzerland) calibrated with Novasina SAL-T humidity standards (33%, 75%, 84%, and 90% relative humidity). Six determinations were carried out for each treatment.

2.3. Analysis of Lipid Oxidation by Thiobarbituric Acid Reactive Substance (TBARS) Measurement

The extent of lipid oxidation was evaluated as thiobarbituric acid reactive substances (TBARS) by the method of Pikul et al. [28]. Thiobarbituric acid (TBA) reacted with malondialdehyde, which resulted in a color compound. The values were expressed as mg of malondialdehyde (MDA) per kilogram of sample. Six determinations were carried out for each treatment.

2.4. Fatty Acid Profile Measurements

Fat extraction from the samples and subsequent transesterification of fatty acids were performed according to Barros et al. [26]. Fatty acid analysis were carried out using a gas chromatograph (GC-Agilent7890B, Agilent Technologies, Santa Clara, CA, USA) following the conditions reported by Barros et al. [29]. Six determinations were carried out for each treatment.

2.5. Volatile Compound Determination

Analysis of volatile compounds was performed in triplicate using the SPME-gas chromatography-mass spectrometry technique (Agilent Technologies, Santa Clara, CA, USA) according to the method described by Pérez-Santaescolástica et al. [30]. The results were expressed as area units per g of sample (AU × 10^4/g of sample). Six determinations were carried out for each treatment.

2.6. Statistical Analysis

The Statistica v. 13.3 software (Dell, Inc., Round Rock, TX, USA) was used to perform the statistical analysis of the results obtained in the experiment. Data were analyzed using a two-way analysis of variance (ANOVA). The significance of the differences between mean values was calculated using Tukey's range *t*-test. All differences were significant at $p \leq 0.05$. The results were expressed as mean ± standard deviation.

3. Results

3.1. Chemical Composition and Physicochemical Properties of Sausages

The proximate chemical composition of the three formulations of beef and fallow deer sausages is shown in Figure 1. As expected, all formulations showed a similar chemical composition in terms of moisture, protein, fat, collagen, and salt contents. Meat beef sausages were characterized by a higher ($p \leq 0.05$) fat content compared to sausages produced from fallow deer meat. The salt concentration in the products was similar due to the same amount added during production and a similar degree of drying of the sausages during processing.

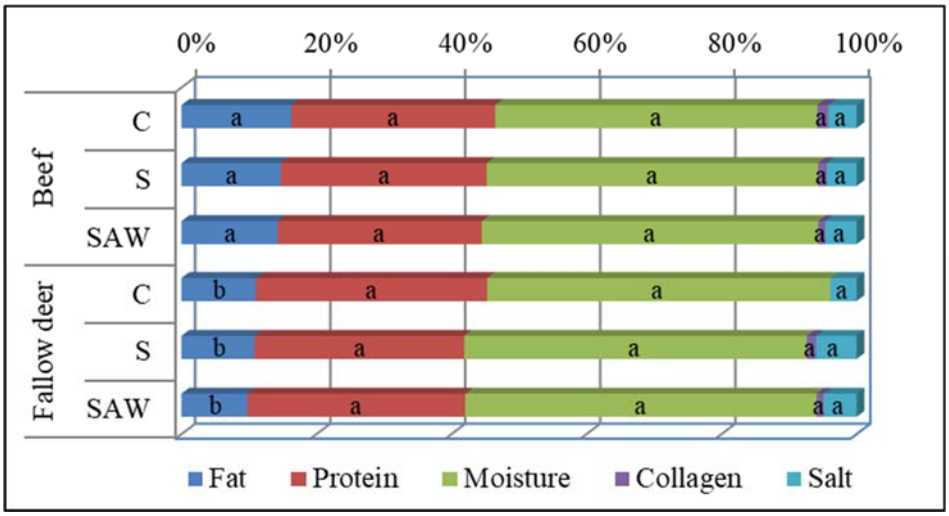

Figure 1. Chemical composition of sausages. C—control sample, S—sample with sea salt, SAW—sample with the addition of sea salt and freeze-dried acid whey (equivalent to 10% of liquid acid whey). [a,b]—Means within one variable followed by the same letters did not differ significantly ($p \leq 0.05$).

Statistical analysis showed that the process factors such as variant and type of meat used influenced ($p \leq 0.05$) the pH, water activity, and TBARS of the tested samples (Table 2). The elimination of nitrite and the addition of acid whey powder had a different effect on the pH of the sausages depending on the type of meat used. In the case of beef sausages, the sample without nitrite with the addition of whey was characterized by the highest pH, while in the case of fallow deer sausages, the same sample (SAW) showed the lowest pH among all the samples. Water activity did not differ statistically significantly between the formulations. The values ranged from 0.910 to 0.915 and from 0.915 to 0.918 for beef and fallow deer fermented sausages, respectively. Regarding lipid oxidation, TBARS values were significantly higher in beef fermented sausages compared to fallow deer sausages. Elimination of nitrite as well as the addition of acid whey powder did not lead to statistically significant changes in TBARS in fermented sausages at the end of production.

Table 2. pH, TBARS, and water activity values of beef and fallow deer fermented sausages (n = 6; mean ± standard deviation).

Parameter	C	S	SAW
pH			
Beef	5.23 ^{d} ± 0.16	5.32 ^{cd} ± 0.03	5.37 ^{c} ± 0.01
Fallow deer	5.74 ^{ab} ± 0.01	5.79 ^{a} ± 0.04	5.66 ^{b} ± 0.01
TBARS (mg MDA/kg)			
Beef	3.38 ^{a} ± 0.09	3.23 ^{a} ± 0.12	3.01 ^{b} ± 0.19
Fallow deer	1.85 ^{c} ± 0.12	1.93 ^{c} ± 0.07	1.99 ^{c} ± 0.06
Water Activity (a_w)			
Beef	0.915 ^{ab} ± 0.008	0.910 ^{b} ± 0.003	0.914 ^{ab} ± 0.003
Fallow deer	0.918 ^{a} ± 0.002	0.918 ^{a} ± 0.002	0.915 ^{ab} ± 0.002

C—Sample with the curing mixture, S—sample with sea salt, SAW—sample with the addition of sea salt and freeze-dried acid whey (equivalent to 10% of liquid acid whey). $^{a–d}$—Means within one variable followed by the same letters did not differ significantly ($p \leq 0.05$).

3.2. Fatty Acid Profile of Sausages

Analysis of the main fractions of fatty acids (Table 3) indicated the effect of the additives used on the SFA, MUFA, and PUFA contents. However, the type of meat used had the greatest impact on the fatty acid profile. Both beef and fallow deer fermented sausages contain a higher proportion of SFA than PUFA. Fallow deer sausages were characterized by about a 15% higher content of saturated fatty acids, which was compensated for by the lower content of monounsaturated fatty acids in comparison to beef sausages (also about 15%). The polyunsaturated fatty acid content was significantly higher in fallow deer sausages compared to beef sausages.

Table 3. Main fractions of the fatty acid profile of fermented sausages made from fallow deer and beef meat with different additives (mean ± standard deviation).

Parameter	Type of Meat	Variant		
		C	S	SAW
SFA (% fatty acid)	Beef	51.72 ^{a} ± 0.80	52.64 ^{b} ± 0.13	52.65 ^{b} ± 0.26
	Fallow deer	65.27 ^{a} ± 0.58	65.38 ^{a} ± 0.54	65.84 ^{a} ± 0.39
MUFA (% fatty acid)	Beef	44.04 ^{a} ± 0.92	42.75 ^{b} ± 0.14	42.75 ^{b} ± 0.24
	Fallow deer	26.46 ^{c} ± 0.93	26.32 ^{c} ± 0.49	25.78 ^{c} ± 0.38
PUFA (% fatty acid)	Beef	4.24 ^{c} ± 0.14	4.61 ^{b} ± 0.08	4.60 ^{b} ± 0.12
	Fallow deer	8.26 ^{a} ± 0.35	8.30 ^{a} ± 0.07	8.38 ^{a} ± 0.12
n-3	Beef	0.71 ^{c} ± 0.03	0.86 ^{b} ± 0.02	0.89 ^{b} ± 0.04
	Fallow deer	2.91 ^{a} ± 0.14	2.92 ^{a} ± 0.02	2.98 ^{a} ± 0.04
n-6	Beef	3.54 ^{b} ± 0.13	3.75 ^{b} ± 0.06	3.71 ^{b} ± 0.09
	Fallow deer	5.36 ^{a} ± 0.22	5.38 ^{a} ± 0.07	5.40 ^{a} ± 0.08
n-6/n-3	Beef	5.02 ^{a} ± 0.24	4.34 ^{b} ± 0.03	4.15 ^{b} ± 0.08
	Fallow deer	1.84 ^{c} ± 0.02	1.84 ^{c} ± 0.03	1.81 ^{c} ± 0.01

C—Sample with the curing mixture, S—sample with sea salt, SAW—sample with the addition of sea salt and freeze-dried acid whey (equivalent to 10% of liquid acid whey). $^{a–c}$—Means within one variable followed by the same letters did not differ significantly ($p \leq 0.05$).

The analysis of the individual fatty acids showed a significant effect of the additives used; however, differences in the fatty acid profile between beef sausages and fallow deer sausages were more evident (Table 4). Beef fermented sausages without nitrite (S, SAW) were characterized by a significantly higher level of C20:4 n-6 and C15:1 n-5 fatty acids compared to the C sample. Compared to fallow deer sausages, beef sausages had a clearly lower contribution of lauric acid (C12:0), myristic acid (C14:0), pentadecanoic acid (C15:0),

heptadecanoic acid (C17:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5), and docosahexaenoic acid (DHA, C22:6) and a higher contribution of palmitoleic (C16:1), elaidic (C18:1), and linolenic (C18:3) acids as well as C14:1 n-5, C15:1 n-5, and C16:1 n-7 fatty acids.

Table 4. Fatty acid profile (%) of sausages made from beef and fallow deer (n = 6; mean ± standard deviation).

Fatty Acid	Type of Meat	Variant		
		C	S	SAW
C10:0	Beef	$0.04^{c} \pm 0.00$	$0.04^{c} \pm 0.00$	$0.05^{b} \pm 0.00$
	Fallow deer	$0.04^{d} \pm 0.00$	$0.04^{d} \pm 0.00$	$0.06^{a} \pm 0.00$
C12:0	Beef	$0.04^{e} \pm 0.00$	$0.06^{d} \pm 0.00$	$0.08^{c} \pm 0.00$
	Fallow deer	$0.13^{b} \pm 0.00$	$0.13^{b} \pm 0.00$	$0.16^{a} \pm 0.00$
C13:0	Beef	nd	nd	nd
	Fallow deer	$0.07^{b} \pm 0.00$	$0.07^{ab} \pm 0.00$	$0.07^{a} \pm 0.00$
C14:0	Beef	$2.39^{d} \pm 0.06$	$2.69^{c} \pm 0.02$	$2.76^{c} \pm 0.05$
	Fallow deer	$3.98^{b} \pm 0.05$	$4.01^{b} \pm 0.07$	$4.17^{a} \pm 0.06$
C14:1 n-5	Beef	$0.35^{b} \pm 0.01$	$0.45^{a} \pm 0.01$	$0.45^{a} \pm 0.01$
	Fallow deer	$0.17^{b} \pm 0.00$	$0.16^{b} \pm 0.00$	$0.17^{b} \pm 0.01$
C15:0	Beef	$0.32^{b} \pm 0.01$	$0.35^{b} \pm 0.00$	$0.30^{b} \pm 0.13$
	Fallow deer	$2.53^{a} \pm 0.11$	$2.51^{a} \pm 0.03$	$2.53^{a} \pm 0.04$
C15:1 n-5	Beef	$0.34^{b} \pm 0.01$	$0.38^{a} \pm 0.01$	$0.38^{a} \pm 0.02$
	Fallow deer	$0.15^{c} \pm 0.01$	$0.14^{c} \pm 0.00$	$0.14^{c} \pm 0.00$
C16:0	Beef	$24.94^{ab} \pm 0.42$	$25.11^{ab} \pm 0.07$	$24.78^{b} \pm 0.17$
	Fallow deer	$24.95^{ab} \pm 0.09$	$25.04^{ab} \pm 0.09$	$25.24^{a} \pm 0.14$
C16:1 n-7	Beef	$2.08^{b} \pm 0.04$	$2.25^{a} \pm 0.03$	$2.21^{a} \pm 0.04$
	Fallow deer	$1.17^{c} \pm 0.04$	$1.18^{c} \pm 0.02$	$1.14^{c} \pm 0.02$
C17:0	Beef	$1.31^{c} \pm 0.02$	$1.35^{bc} \pm 0.01$	$1.35^{b} \pm 0.01$
	Fallow deer	$1.96^{a} \pm 0.02$	$1.97^{a} \pm 0.03$	$1.98^{a} \pm 0.01$
C17:1 n-7	Beef	$0.62^{b} \pm 0.02$	$0.64^{a} \pm 0.01$	$0.62^{ab} \pm 0.01$
	Fallow deer	$0.36^{d} \pm 0.01$	$0.39^{c} \pm 0.01$	$0.38^{cd} \pm 0.01$
C18:0	Beef	$22.42^{c} \pm 0.34$	$22.79^{bc} \pm 0.13$	$23.07^{b} \pm 0.19$
	Fallow deer	$30.73^{a} \pm 0.40$	$30.73^{a} \pm 0.32$	$30.73^{a} \pm 0.17$
C18:1 *trans11*	Beef	$0.50^{b} \pm 0.55$	nd	nd
	Fallow deer	$2.00^{a} \pm 0.07$	$2.00^{a} \pm 0.04$	$2.07^{a} \pm 0.03$
C18:1 n-9	Beef	$38.81^{a} \pm 0.49$	$37.71^{b} \pm 0.17$	$37.78^{b} \pm 0.26$
	Fallow deer	$21.69^{c} \pm 0.89$	$21.49^{c} \pm 0.45$	$20.93^{c} \pm 0.35$
C18:1 n-7	Beef	$1.10^{a} \pm 0.03$	$1.07^{a} \pm 0.03$	$1.05^{a} \pm 0.03$
	Fallow deer	$0.76^{b} \pm 0.06$	$0.81^{b} \pm 0.01$	$0.79^{b} \pm 0.01$
C18:2 *trans9-trans11*	Beef	$0.16^{c} \pm 0.02$	$0.18^{b} \pm 0.00$	$0.18^{b} \pm 0.00$
	Fallow deer	$0.26^{a} \pm 0.01$	$0.26^{a} \pm 0.00$	$0.27^{a} \pm 0.01$
C18:2 *cis9-trans11*	Beef	$0.32^{b} \pm 0.02$	$0.39^{a} \pm 0.01$	$0.39^{a} \pm 0.01$
	Fallow deer	$0.24^{c} \pm 0.01$	$0.24^{c} \pm 0.00$	$0.24^{c} \pm 0.00$
C18:2 n-6	Beef	$2.57^{b} \pm 0.09$	$2.62^{b} \pm 0.03$	$2.56^{b} \pm 0.04$
	Fallow deer	$3.45^{a} \pm 0.13$	$3.48^{a} \pm 0.05$	$3.48^{a} \pm 0.05$
C18:3 n-3 (ALA)	Beef	$0.49^{d} \pm 0.04$	$0.55^{c} \pm 0.01$	$0.55^{c} \pm 0.02$
	Fallow deer	$1.20^{b} \pm 0.04$	$1.22^{ab} \pm 0.01$	$1.25^{a} \pm 0.01$
C20:0	Beef	$0.17^{b} \pm 0.00$	$0.17^{b} \pm 0.00$	$0.17^{b} \pm 0.00$
	Fallow deer	$0.47^{a} \pm 0.02$	$0.48^{a} \pm 0.02$	$0.48^{a} \pm 0.01$

Table 4. *Cont.*

Fatty Acid	Type of Meat	Variant		
		C	S	SAW
C20:1 n-9	Beef	0.23 ^{a} ± 0.00	0.23 ^{ab} ± 0.00	0.23 ^{b} ± 0.00
	Fallow deer	0.17 ^{c} ± 0.01	0.16 ^{d} ± 0.00	0.16 ^{cd} ± 0.00
C20:2 n-6	Beef	0.05 ^{cd} ± 0.01	0.04 ^{d} ± 0.00	0.04 ^{d} ± 0.00
	Fallow deer	0.09 ^{a} ± 0.00	0.08 ^{ab} ± 0.01	0.06 ^{bc} ± 0.00
C20:3 n-6	Beef	0.11 ^{a} ± 0.01	0.12 ^{a} ± 0.00	0.12 ^{a} ± 0.00
	Fallow deer	0.05 ^{c} ± 0.00	0.06 ^{b} ± 0.00	0.06 ^{bc} ± 0.01
C20:3 n-3	Beef	0.03 ^{b} ± 0.00	0.03 ^{b} ± 0.00	0.03 ^{b} ± 0.00
	Fallow deer	0.05 ^{a} ± 0.00	0.05 ^{a} ± 0.00	0.05 ^{a} ± 0.00
C20:4 n-6	Beef	0.32 ^{c} ± 0.02	0.40 ^{b} ± 0.01	0.42 ^{b} ± 0.02
	Fallow deer	1.27 ^{a} ± 0.09	1.26 ^{a} ± 0.01	1.29 ^{a} ± 0.03
C20:5 n-3	Beef	0.05 ^{c} ± 0.00	0.10 ^{b} ± 0.00	0.12 ^{b} ± 0.01
	Fallow deer	0.38 ^{a} ± 0.02	0.37 ^{a} ± 0.00	0.38 ^{a} ± 0.01
C21:0	Beef	0.05 ^{b} ± 0.01	0.05 ^{b} ± 0.00	0.05 ^{b} ± 0.00
	Fallow deer	0.11 ^{a} ± 0.01	0.11 ^{a} ± 0.01	0.11 ^{a} ± 0.01
C22:0	Beef	0.02 ^{b} ± 0.00	0.02 ^{b} ± 0.00	0.02 ^{b} ± 0.00
	Fallow deer	0.15 ^{a} ± 0.01	0.14 ^{a} ± 0.01	0.14 ^{a} ± 0.01
C22:1 n-9	Beef	0.02 ^{a} ± 0.02	0.03 ^{a} ± 0.00	0.03 ^{a} ± 0.00
	Fallow deer	nd	nd	nd
C22:5 n-3	Beef	0.12 ^{b} ± 0.01	0.16 ^{b} ± 0.01	0.16 ^{b} ± 0.01
	Fallow deer	1.17 ^{a} ± 0.07	1.18 ^{a} ± 0.02	1.20 ^{a} ± 0.02
C22:6 n-3 (DHA)	Beef	0.02 ^{b} ± 0.01	0.03 ^{b} ± 0.00	0.03 ^{b} ± 0.00
	Fallow deer	0.11 ^{a} ± 0.01	0.11 ^{a} ± 0.01	0.11 ^{a} ± 0.00
C23:0	Beef	0.01 ^{b} ± 0.01	0.01 ^{b} ± 0.00	0.02 ^{b} ± 0.00
	Fallow deer	0.09 ^{a} ± 0.01	0.09 ^{a} ± 0.00	0.09 ^{a} ± 0.00
C24:0	Beef	nd	nd	nd
	Fallow deer	0.07 ^{a} ± 0.01	0.07 ^{a} ± 0.00	0.07 ^{a} ± 0.00

nd—not detected. Variant: C—sample with the addition of the curing mixture; S—sample with the addition of sea salt; SAW—sample with the addition of sea salt and freeze-dried acid whey (equivalent to 10% of liquid acid whey). $^{a–d}$—Means within one variable followed by the same letters did not differ significantly ($p \leq 0.05$).

3.3. Volatile Compounds of Sausages

A total of 63 volatile compounds were identified in fermented sausages after processing (Tables 5–7). These compounds were grouped according to their possible origin, in order to understand how generation pathways were affected by the fermentation process. Three major groups were assigned: lipid auto-oxidation products (16), products of bacterial metabolism (21), and unknown origin (26). Within the second group, four subgroups were named: carbohydrate fermentation (6), amino acid degradation (6), staphylococci esterase activity (7) and lipid β-oxidation (2). Several of the compounds listed may have more than one origin. Volatile compounds derived from lipid oxidation of beef and fallow deer sausages are shown in Table 5. These compounds include mostly aldehydes and alcohols, which are considered as markers of secondary oxidation of fatty acids. The most abundant compounds derived from lipid oxidation were hexanal and 1-hexanol. Fallow deer fermented sausages were characterized by lower values of lipid auto oxidation-derived volatile compounds, although the differences in many cases were not statistically significant. The elimination of nitrite and addition of acid whey powder (SAW) resulted in a reduction in the hexanal content compared to the sample with nitrite (C).

Table 5. Lipid auto oxidation-derived volatile compounds (AU-EIC $\times 10^4$/g of sample) of sausages made from beef and fallow deer (n = 6; mean ± standard deviation).

	Type of Meat	Variant		
		C	S	SAW
1-Propanol	Beef	23.46 ^{ab} ± 15.03	39.04 ^{a} ± 32.62	23.50 ^{ab} ± 17.63
	Fallow deer	3.20 ^{b} ± 0.72	4.41 ^{b} ± 0.69	6.19 ^{b} ± 2.15
Heptane	Beef	280.84 ^{a} ± 106.39	331.95 ^{a} ± 85.74	208.95 ^{a} ± 101.89
	Fallow deer	21.48 ^{b} ± 9.75	42.87 ^{b} ± 18.53	32.08 ^{b} ± 11.14
Pentanal	Beef	78.65 ^{a} ± 65.12	49.57 ^{ab} ± 19.90	9.53 ^{b} ± 4.44
	Fallow deer	2.69 ^{b} ± 1.42	6.19 ^{b} ± 2.36	1.70 ^{b} ± 0.72
1-Pentanol	Beef	447.75 ^{ab} ± 272.87	622.25 ^{a} ± 483.45	394.17 ^{ab} ± 240.36
	Fallow deer	14.56 ^{b} ± 1.98	64.59 ^{b} ± 11.76	124.37 ^{b} ± 25.24
Hexanal	Beef	1561.07 ^{a} ± 1333.82	1032.11 ^{ab} ± 435.34	102.26 ^{b} ± 52.75
	Fallow deer	30.92 ^{b} ± 7.82	90.97 ^{b} ± 36.51	22.66 ^{b} ± 6.92
n-Hexane	Beef	361.01 ^{ab} ± 277.77	276.30 ^{b} ± 224.66	679.92 ^{ab} ± 303.73
	Fallow deer	981.14 ^{a} ± 585.05	827.80 ^{ab} ± 774.92	750.46 ^{ab} ± 391.11
Heptanal	Beef	797.69^{a} ± 624.45	794.27 ^{a} ± 584.61	76.19 ^{b} ± 42.52
	Fallow deer	6.55 ^{b} ± 2.35	11.41 ^{b} ± 1.59	6.31 ^{b} ± 1.84
Octanal	Beef	153.78 ^{ab} ± 139.17	170.25 ^{a} ± 160.88	19.44 ^{ab} ± 7.04
	Fallow deer	5.50 ^{b} ± 2.64	4.98 ^{ab} ± 1.15	4.26 ^{b} ± 1.86
Hexanoic acid	Beef	58.98 ^{b} ± 44.94	61.85 ^{b} ± 39.83	4.30 ^{b} ± 2.53
	Fallow deer	16.77 ^{b} ± 5.48	24.23 ^{b} ± 4.95	378.13 ^{a} ± 54.56
Nonanal	Beef	106.43 ^{ab} ± 82.35	129.88 ^{a} ± 104.09	33.43 ^{abc} ± 16.35
	Fallow deer	5.84 ^{c} ± 2.88	6.29 ^{bc} ± 0.70	2.59 ^{c} ± 0.72
2-Nonenal, (E)-	Beef	14.06 ^{ab} ± 12.81	16.28 ^{a} ± 13.32	5.60 ^{ab} ± 3.02
	Fallow deer	0.85 ^{b} ± 0.47	1.18 ^{ab} ± 1.48	4.29 ^{ab} ± 3.05
1-Butanol	Beef	38.22 ^{a} ± 11.00	42.16 ^{a} ± 13.42	33.51 ^{ab} ± 11.58
	Fallow deer	14.99 ^{c} ± 4.11	12.82 ^{c} ± 2.03	21.07 ^{bc} ± 3.73
1-Hexanol	Beef	1830.27 ^{ab}± 1370.87	3170.59 ^{a} ± 2544.72	1680.55 ^{ab} ± 851.03
	Fallow deer	25.13 ^{b} ± 3.66	56.95 ^{b} ± 9.43	313.18 ^{b} ± 28.45
Furan, 2-pentyl-	Beef	17.28 ^{ab} ± 11.23	32.28 ^{a} ± 24.00	14.71 ^{ab} ± 6.58
	Fallow deer	2.68 ^{b} ± 0.70	4.00 ^{b} ± 0.73	6.63 ^{b} ± 0.86
Furan, 2-ethyl-	Beef	48.98 ^{abc} ± 26.15	98.68 ^{a} ± 64.00	72.70 ^{ab} ± 45.62
	Fallow deer	3.53 ^{c} ± 0.78	9.06 ^{c} ± 1.64	27.19 ^{bc} ± 8.60
Pentane	Beef	3.60 ^{a} ± 1.44	5.07 ^{a} ± 1.48	3.23 ^{a} ± 1.30
	Fallow deer	0.38 ^{b} ± 0.20	1.15 ^{b} ± 0.40	1.34 ^{b} ± 0.58

Variant: C—sample with the addition of the curing mixture; S—sample with the addition of sea salt; SAW—sample with the addition of sea salt and freeze-dried acid whey (equivalent to 10% of liquid acid whey). $^{a–c}$—Means within one variable followed by the same letters did not differ significantly ($p \leq 0.05$).

Carbohydrate-fermentation derived volatiles were the most abundant group of bacterial metabolism-derived volatile compounds in all the experimental sausage samples, while those derived from lipid β-oxidation represented the least abundant group of bacterial metabolism-derived volatile compounds (Table 6). Taking into account the type of meat, fallow deer sausages were characterized by significantly higher 2-Butanol, acetoin, 1-Butanol, and 3-methyl- and significantly lower phenyl acetaldehyde contents compared to beef sausages. Samples of beef fermented sausage without nitrite with acid whey powder (SAW) had the lowest contents of acetic acid and 3-Methyl-butanal and the highest contents of 1-Butanol, 3-methyl-, phenyl ethyl alcohol, and 2-Pentanone. The most abundant bacterial metabolism-derived volatile compound was 2,3-Butanediol, [R-(R*,R*)]—for both beef and fallow deer sausages. Its highest amount was recorded in the samples C and S.

Table 6. Bacterial metabolism-derived volatile compounds (AU-EIC $\times 10^4$/g of sample) in sausages made from beef and fallow deer (n = 6; mean ± standard deviation).

	Type of Meat	Variant		
		C	S	SAW
Carbohydrate fermentation				
2-Butanone	Beef	3.47 ^{b} ± 0.79	6.67 ^{b} ± 2.78	11.05 ^{b} ± 5.06
	Fallow deer	94.76 ^{ab} ± 28.60	270.39 ^{a} ± 231.98	31.85 ^{ab} ± 4.49
2-Butanol	Beef	4.26 ^{c} ± 2.43	4.82 ^{c} ± 1.10	9.21 ^{c} ± 1.47
	Fallow deer	91.61 ^{a} ± 10.44	76.29 ^{ab} ± 54.09	39.46 ^{b} ± 8.54
Acetic acid	Beef	1609.96 ^{a} ± 376.13	1843.73 ^{a} ± 160.02	831.99 ^{b} ± 467.98
	Fallow deer	964.29 ^{b} ± 176.10	1013.81 ^{b} ± 349.62	905.72 ^{b} ± 191.66
Acetoin	Beef	302.51 ^{c} ± 138.27	267.57 ^{cd} ± 31.74	321.09 ^{c} ± 60.61
	Fallow deer	706.53 ^{b} ± 137.28	1080.88 ^{a} ± 237.54	74.73 ^{d} ± 27.70
2,3-Butanediol, [R (R*,R*)]-	Beef	1994.05 ^{ab} ± 39.8	2504.90 ^{a} ± 1115.46	1191.95 ^{bc} ± 618.97
	Fallow deer	1319.46 ^{bc} ± 119.36	1378.20 ^{bc} ± 289.11	829.16 ^{c} ± 249.57
Butanoic acid	Beef	206.93 ^{c} ± 30.99	238.65 ^{c} ± 20.38	287.36 ^{bc} ± 77.80
	Fallow deer	316.70 ^{bc} ± 46.33	450.25 ^{b} ± 72.28	1097.02 ^{a} ± 130.67
Staphylococci esterase activity				
Ethyl acetate	Beef	111.04 ^{cd} ± 25.72	108.81 ^{d} ± 25.82	170.63 ^{bc} ± 37.40
	Fallow deer	254.08 ^{ab} ± 56.89	143.80 ^{b} ± 76.66	194.02 ^{ab} ± 15.59
Ethyl (S)-(-)-lactate	Beef	224.06 ^{ab} ± 22.50	255.54 ^{a} ± 3.71	263.39 ^{a} ± 80.54
	Fallow deer	147.67 ^{bc} ± 86.44	88.10 ^{c} ± 74.83	219.52 ^{ab} ± 18.31
Ethyl 3-methyl-butanoate	Beef	403.80 ^{a} ± 54.41	294.83 ^{ab} ± 21.56	287.5 ^{ab} ± 31.98
	Fallow deer	228.12 ^{b} ± 104.91	225.45 ^{ab} ± 168.38	212.85 ^{b} ± 10.79
Ethyl butanoate	Beef	205.54 ^{d} ± 19.02	194.32 ^{d} ± 41.11	277.24 ^{cd} ± 59.28
	Fallow deer	610.59 ^{b} ± 324.16	414.52 ^{bc} ± 336.97	1087.47 ^{a} ± 103.32
Ethyl octanoate	Beef	4.20 ^{b} ± 1.88	3.88 ^{b} ± 1.03	6.03 ^{b} ± 1.66
	Fallow deer	9.43 ^{b} ± 4.56	7.33 ^{b} ± 5.96	59.78 ^{a} ± 9.20
Ethyl hexanoate	Beef	269.60 ^{b} ± 184.75	303.25 ^{b} ± 188.08	128.34 ^{b} ± 22.93
	Fallow deer	95.52 ^{b} ± 49.14	94.67 ^{b} ± 60.91	529.20 ^{a} ± 92.04
Ethyl 4-methylpentanoate	Beef	1.91 ^{b} ± 0.37	1.90 ^{b} ± 0.17	1.39 ^{b} ± 0.34
	Fallow deer	45.85 ^{a} ± 25.09	33.20 ^{a} ± 26.61	54.64 ^{a} ± 8.03
Amino acid degradation				
3-Methyl-butanal	Beef	95.23 ^{a} ± 72.26	92.84 ^{a} ± 71.78	5.78 ^{b} ± 2.61
	Fallow deer	33.71 ^{ab} ± 10.48	111.94 ^{a} ± 26.44	20.01 ^{ab} ± 5.80
1-Butanol, 3-methyl-	Beef	701.89 ^{b} ± 150.92	700.98 ^{b} ± 102.83	1184.91 ^{a} ± 191.61
	Fallow deer	72.02 ^{c} ± 10.08	116.03 ^{c} ± 61.54	51.12 ^{c} ± 7.68
Phenyl acetaldehyde	Beef	330.96 ^{d} ± 233.16	225.21 ^{de} ± 123.31	16.18 ^{e} ± 5.55
	Fallow deer	900.85 ^{c} ± 57.39	1288.59 ^{b} ± 215.24	1780.05 ^{a} ± 133.42
Phenyl ethyl alcohol	Beef	416.73 ^{bc} ± 186.70	560.51 ^{b} ± 241.41	1322.63 ^{a} ± 209.22
	Fallow deer	97.22 ^{d} ± 29.28	188.30 ^{cd} ± 113.36	432.77 ^{bc} ± 177.22
Benzaldehyde	Beef	169.4 ^{ab} ± 115.03	209.63 ^{a} ± 82.83	70.25 ^{b} ± 15.37
	Fallow deer	61.10 ^{a} ± 19.07	173.29 ^{ab} ± 80.07	204.69 ^{a} ± 61.18
Dimethyl trisulfide	Beef	13.30 ^{ab} ± 11.11	16.26^{a} ± 6.54	4.58 ^{bc} ± 3.78
	Fallow deer	0.11 ^{c} ± 0.00	1.20 ^{c} ± 1.11	0.45 ^{c} ± 0.31
Lipid β-oxidation				
2-Pentanone	Beef	3.74 ^{b} ± 1.52	5.38 ^{b} ± 1.55	25.57 ^{a} ± 8.71
	Fallow deer	1.75 ^{b} ± 0.49	2.31 ^{b} ± 0.90	3.16 ^{b} ± 0.84
2,3-Pentanedione	Beef	39.36 ^{ab} ± 33.89	41.96 ^{a} ± 37.07	5.90 ^{b} ± 3.50
	Fallow deer	2.44 ^{b} ± 1.09	3.65 ^{b} ± 0.76	3.30 ^{b} ± 1.70

Variant: C—sample with the addition of the curing mixture; S—sample with the addition of sea salt; SAW—sample with the addition of sea salt and freeze-dried acid whey (equivalent to 10% of liquid acid whey). $^{a–d}$—Means within one variable followed by the same letters did not differ significantly ($p \leq 0.05$).

Table 7. Volatile compounds (AU-EIC $\times 10^4$/g of sample) of unknown origin in sausages made from beef and fallow deer (n = 6; mean ± standard deviation).

	Type of Meat	Variant		
		C	S	SAW
1,3-Benzenediol, monobenzoate	Beef	49.61 ^{bc} ± 8.18	56.14 ^{bc} ± 11.00	43.52 ^{c} ± 6.10
	Fallow deer	48.16 ^{bc} ± 3.33	61.71 ^{ab} ± 9.45	71.74 ^{a} ± 7.93
1-Butanol, 3-methyl-, acetate	Beef	22.11 ^{ab} ± 7.38	20.73 ^{b} ± 7.52	31.11 ^{a} ± 8.02
	Fallow deer	3.91 ^{c} ± 0.69	7.65 ^{c} ± 4.43	3.54 ^{c} ± 0.38
2-Butanol, (R)-	Beef	2.76 ^{d} ± 0.83	3.04 ^{d} ± 0.85	4.05 ^{cd} ± 0.36
	Fallow deer	34.77 ^{a} ± 5.89	29.28 ^{ab} ± 19.40	15.96 ^{bc} ± 2.42
2-Propen-1-amine	Beef	24.86 ^{b} ± 9.97	26.24 ^{b} ± 15.59	55.52 ^{a} ± 15.22
	Fallow deer	5.68 ^{c} ± 0.80	6.64 ^{bc} ± 2.17	5.69 ^{c} ± 1.09
4-Pentenoic acid, 2,2-diethyl-3-oxo-5-phenyl-, ethyl ester	Beef	31.20 ^{ab} ± 11.45	38.55 ^{b} ± 17.33	14.20 ^{bc} ± 9.26
	Fallow deer	17.95 ^{bc} ± 6.49	29.95 ^{abc} ± 7.53	10.38 ^{c} ± 5.08
6-Fluoro-2-trifluoromethylbenzoic acid, 2,3-dichlorophenyl ester	Beef	90.63 ^{a} ± 27.86	79.41 ^{a} ± 30.71	83.62 ^{a} ± 17.40
	Fallow deer	71.98 ^{a} ± 33.78	73.67 ^{a} ± 22.02	85.40 ^{a} ± 25.23
Acetic acid ethenyl ester	Beef	9.85 ^{c} ± 4.85	7.67 ^{c} ± 3.05	8.89 ^{c} ± 4.25
	Fallow deer	36.76 ^{b} ± 11.98	75.71 ^{a} ± 31.86	2.07 ^{c} ± 1.13
Benzene, 1,3-dimethyl-	Beef	25.31 ^{ab} ± 6.84	29.82 ^{a} ± 2.54	29.67 ^{a} ± 4.56
	Fallow deer	18.76 ^{bc} ± 5.46	16.19 ^{c} ± 5.95	17.59 ^{bc} ± 3.80
Benzyl alcohol	Beef	241.08 ^{c} ± 67.46	348.67 ^{ab} ± 56.32	360.14 ^{a} ± 48.95
	Fallow deer	134.22 ^{d} ± 16.49	187.36 ^{cd} ± 18.14	274.72 ^{bc} ± 34.18
Carbon disulphide	Beef	314.17 ^{abc} ± 258.96	597.50 ^{a} ± 209.74	475.77 ^{ab} ± 223.76
	Fallow deer	118.48 ^{c} ± 40.17	369.16 ^{abc} ± 144.92	276.52 ^{bc} ± 95.46
Cyclobutanol	Beef	12.70 ^{ab} ± 10.78	21.72 ^{a} ± 17.36	20.72 ^{ab} ± 11.92
	Fallow deer	6.72 ^{ab} ± 2.54	8.68 ^{ab} ± 1.62	3.19 ^{b} ± 4.62
Cyclopentanol	Beef	102.05 ^{ab} ± 67.20	131.71 ^{a} ± 62.18	52.09 ^{bc} ± 15.78
	Fallow deer	7.21 ^{c} ± 1.07	20.95 ^{c} ± 4.59	38.26 ^{bc} ± 6.03
Dimethyl sulfone	Beef	38.72 ^{b} ± 7.84	72.89 ^{a} ± 33.88	37.70 ^{b} ± 17.43
	Fallow deer	59.40 ^{ab} ± 5.34	67.95 ^{ab} ± 14.98	40.89 ^{b} ± 10.59
Ethanedioic acid, dibutyl ester	Beef	189.73 ^{ab} ± 47.86	239.55 ^{a} ± 62.01	150.86 ^{b} ± 29.66
	Fallow deer	21.42 ^{c} ± 8.77	46.25 ^{c} ± 14.09	25.82 ^{c} ± 11.97
Ethanol, 2-methoxy-, acetate	Beef	14.41 ^{ab} ± 7.44	20.51 ^{a} ± 12.64	12.78 ^{ab} ± 5.81
	Fallow deer	0.59 ^{c} ± 0.19	3.15 ^{bc} ± 0.68	4.38 ^{bc} ± 1.32
Formic acid, heptyl ester	Beef	295.98 ^{ab} ± 239.27	647.20 ^{a} ± 582.33	336.18 ^{ab} ± 278.27
	Fallow deer	4.26 ^{b} ± 1.04	6.01 ^{b} ± 0.95	22.23 ^{b} ± 7.84
Formic acid, octyl ester	Beef	42.80 ^{ab} ± 30.64	74.47 ^{a} ± 61.04	32.07 ^{ab} ± 17.35
	Fallow deer	2.55 ^{c} ± 0.94	2.22 ^{c} ± 1.05	5.21 ^{c} ± 0.99
Glycidol	Beef	37.24 ^{ab} ± 22.84	37.41 ^{ab} ± 29.62	2.11 ^{c} ± 0.97
	Fallow deer	13.68 ^{abc} ± 4.83	45.76 ^{a} ± 8.40	9.04 ^{c} ± 3.40
Heptane, 2,2,4,6,6-pentamethyl-	Beef	63.59 ^{a} ± 45.60	61.02 ^{a} ± 40.76	68.04 ^{a} ± 41.59
	Fallow deer	82.31 ^{a} ± 36.54	56.40 ^{a} ± 33.07	96.73 ^{a} ± 49.77
Heptane, 2,4-dimethyl-	Beef	699.66 ^{b} ± 106.94	1021.40 ^{a} ± 152.05	626.03 ^{b} ± 47.67
	Fallow deer	76.21 ^{c} ± 18.77	136.40 ^{c} ± 35.72	143.81 ^{c} ± 29.99
Methane, dichloronitro-	Beef	20.87 ^{a} ± 6.55	18.80 ^{a} ± 6.83	25.48 ^{a} ± 6.49
	Fallow deer	30.93 ^{a} ± 13.13	33.57 ^{a} ± 27.14	30.70 ^{a} ± 15.09
Methane, trimethoxy-	Beef	138.00 ^{ab} ± 28.89	177.13 ^{a} ± 78.27	86.29 ^{b} ± 46.38
	Fallow deer	97.05 ^{b} ± 10.35	105.98 ^{ab} ± 21.92	67.01 ^{b} ± 18.92
Octane, 2,2,6-trimethyl-	Beef	36.36 ^{a} ± 35.76	2.22 ^{b} ± 0.91	14.27 ^{ab} ± 11.82
	Fallow deer	0.79 ^{b} ± 0.49	1.00 ^{b} ± 0.15	14.94 ^{ab} ± 2.89
Oxirane, (methoxymethyl)-	Beef	3.81 ^{a} ± 3.69	9.56 ^{a} ± 9.65	8.94 ^{a} ± 10.84
	Fallow deer	0.86 ^{a} ± 0.68	1.07 ^{a} ± 0.37	2.33 ^{a} ± 1.64
Pentanoic acid, 2-methyl-, anhydride	Beef	9.89 ^{bc} ± 11.44	16.23 ^{a} ± 17.17	2.90 ^{bc} ± 1.94
	Fallow deer	0.30 ^{c} ± 0.28	0.27 ^{bc} ± 0.16	0.94 ^{bc} ± 0.33
Pyrolo [3,2-d] pyrimidin-2,4(1H,3H)-dione	Beef	643.83 ^{a} ± 60.84	580.02 ^{a} ± 82.22	345.80 ^{bc} ± 92.02
	Fallow deer	415.06 ^{b} ± 58.62	308.45 ^{c} ± 46.30	309.95 ^{bc} ± 34.01

Variant: C—sample with the addition of the curing mixture; S—sample with the addition of sea salt; SAW—sample with the addition of sea salt and freeze-dried acid whey (equivalent to 10% of liquid acid whey). $^{a–d}$—Means within one variable followed by the same letters did not differ significantly ($p \leq 0.05$).

A total of 26 compounds not included in the previous groups were detected in fermented sausages (Table 7). The contents of these compounds depended on both the type of meat and the additives used. The highest amount of heptane, 2,4-dimethyl was measured in beef sausages.

4. Discussion

The latest trends in the composition of fermented meat products are directed to the reduction of additives such as nitrifying agents, which include nitrates and nitrites. Our previous studies [12–14] on selected parameters related to food safety and the bioactive compound content in fallow deer and beef fermented sausages without nitrite showed that the addition of freeze-dried acid whey powder improved the CLA content of meat products, thereby improving their nutritional value. Moreover, the use of freeze-dried acid whey allows obtaining non-nitrite fermented sausages with similar parameters related to food safety as cured sausages, including the content of bioactive amines. Of the three levels of whey added to fermented sausages used in the above-mentioned studies, the equivalent of 10% liquid acid whey, corresponding to 0.7% freeze-dried whey powder, seems to be the most advantageous. The present study using this amount of whey powder for fermented sausages showed a significant effect on the volatiles profile. The products with the material tested in this experiment were characterized by an average pH in the range of 5.23–5.37 and 5.66–5.79 for beef and fallow deer fermented sausages, respectively. Their water activity at the end of production was relatively low (0.910–0.918). The observed pH and water activity parameters indicated unfavorable conditions preventing the growth of the most abundant bacteria in meat products such as *Listeria monocytogenes*, *Clostridium botulinum*, and *Escherichia coli*. Elimination of nitrite as well as the addition of freeze-dried acid whey powder did not cause statistically significant changes in the values of TBARS in fermented sausages at the end of production. TBARS refersto the content of substances that react with thiobarbituric acid, mainly malondialdehyde. Malondialdehyde is one of the most abundant aldehydes generated during secondary lipid oxidation and also probably the most commonly used as an oxidation marker [31]. It is a reactive aldehyde that forms interactions with nucleic acids and covalent protein adducts, thus contributing to its toxicity [32], especially in meat and meat products. It is usually considered a biomarker of oxidative stress in an organism. Covalent modification of lipoproteins with MDA may play a pathogenic role in atherosclerosis [33]. According to the literature [34], the acceptable limit of TBARS in which there is no rancidity in meat and meat products is 2.0–2.5 mg MDA/kg. In the current study, beef fermented sausages were characterized by slightly higher values of TBARS than the indicated limits and significantly higher compared to fallow deer sausages. This may be due to the higher content of unsaturated fatty acids particularly susceptible to oxidation processes in beef fermented sausages as shown by the analysis of the fatty acid profile. Moreover, analysis of the main fractions of fatty acids indicated the effect of the elimination of nitrite on the higher content of PUFA in case of beef sausages. However, beef sausages were characterized by a significantly lower content of n-3 fatty acids, which resulted in the n-6 to n-3 ratio for these sausages being significantly higher compared to the fallow deer sausages. Although the ratio of n-6 to n-3 fatty acids for both beef and fallow deer sausages for human diets was in line with the recommendations [35], for fallow deer sausages it was more than two times lower and amounted to 1.8:1, which corresponds to the ideal ratio of around 2:1 recommended by a panel of lipid experts.

The results obtained displayed a similar relationship of the values of TBARS in comparison to those obtained by Kononiuk and Karwowska [36]. In their study, fallow deer sausages were characterized by a lower content of 2-thiobarbituric acid reactive substances than sausages made from beef meat. The higher values of TBARS are in accordance with the abundance of volatile compounds derived from lipid oxidation as they were significantly higher in beef fermented sausages. As Corral et al. [37] noted, due to their low olfactory threshold, volatile compounds derived from lipid autooxidation are considered essential

contributors to the characteristic aroma of dry fermented sausages. According to Soncin et al. [38], the main fraction of lipids responsible for the formation of specific volatiles includes phospholipids, and to a lesser extent triacylglycerols. This specificity is due to the fact that phospholipids contain more unsaturated fatty acids compared to the acids occurring in triacylglycerols. As described by Arshad et al. [39], phospholipids contain relatively high amounts of linolenic and arachidic acids that are subject to auto-oxidation processes, which result in the formation of: 2,4-decadienal, 2-nonenal, 1-octen-3-one, and 2,4-nonadienal. The lipid oxidation-derived volatile compounds in current studies on fermented sausages include compounds such as alkenals, aldehydes, and ketones, similar to the study by Vargas-Ramella et al. [40]. Concerning the type of meat, beef sausages were characterized by a higher amount of hexanal, which gives a green, grassy odor [41] compared to fallow deer sausages. Hexanal is a typical volatile compound of oxidizing linoleic acid similar to pentanal and octanal [24]. The results of the research carried out by Dominguez et al. [42] showed that dry cured ham was characterized by the highest content of aldehydes (pentanal, hexanal, octanal) among the various fermented meat products. As shown by the results of the analysis of the fatty acid profile, beef sausages had a lower content of linoleic acid compared to the fallow deer product, which may indicate the ongoing oxidation processes. The content of all volatile compounds derived from fat oxidation did not differ significantly between the samples with and without the addition of nitrite (C and S). On the contrary, the results obtained by Marco et al. [41] showed higher amounts of propanal, butanal, 1-penten-3-one, pentanal, heptanal, pentanoic acid, 2-pentyl-furan, 1-octen-3-one, octanal, hexanoic acid, 2-octenal, and 1-octanol in samples with added nitrite. Similarly, research by Perea-Sanz et al. [43] indicated that 25% nitrate reduction increased the heptanal content in dry sausages.

Another group of volatile compounds present in beef and fallow deer fermented sausages are those derived from bacterial metabolism. Lactic acid bacteria and staphylococci generate volatile compounds from amino acid degradation and carbohydrate fermentation while staphylococci additionally generate ethyl esters through their esterase activity [17,22,44,45]. Carbohydrate fermentation-derived volatiles were the most abundant group of bacterial metabolism-derived volatile compounds in the experimental fermented sausages. These results are in accordance with those reported by Perea-Sanz et al. [43]; however, they found that among this group of compounds (acetic acid and ethanol) were the most abundant compounds, whereas in the present study, the most abundant compound in the group of carbohydrate fermentation-derived volatiles was 2,3-Butanediol, [R-(R*,R*)]-. Similar to our findings, 2,3-butanediol was quantified as the dominant alcohol in Turkish sausages obtained from beef [46]. As reported by Luo et al. [46], 2,3-Butanediol is formed by the reduction of methyl ketones from the α-oxidation of fatty acids and is often detected in fermented jerky.

Volatile compounds derived from esterase activity in the current study included seven compounds. Ethyl butanoate and ethyl 3-methyl-butanoate were the most abundant in both beef and fallow deer fermented sausages. Elimination of nitrite had no impact on the production of these volatile compounds; however, the addition of freeze-dried whey powder to uncured sausages resulted in increasing ethyl (S)—(-)-lactate, ethyl butanoate, and ethyl hexanoate during the production of fallow deer fermented sausage. The origin of ester compounds in traditional fermented sausages can be due to different microbial groups including lactic acid bacteria, coagulase-negative cocci, yeast, and mold. Staphylococci are known to esterify alcohols and acids that are present in the microorganism environment. Among the volatile compounds analyzed in dry sausages, ester compounds contribute to the fruity aroma notes associated with a high acceptance of traditional dry sausages [47].

Concerning volatile compounds derived from amino acid degradation, in general, no effect of elimination of nitrite was found, although statistically significant differences were found for several compounds in the uncured sample with the addition of freeze-dried whey powder, similar to the results of other volatile compounds derived from bacterial metabolism. Beef fermented sausage with whey powder addition (SAW) was characterized

by a higher amount of phenyl ethyl alcohol and 1-Butanol, 3-methyl while the highest phenyl acetaldehyde content was found for fallow deer sausage with freeze-dried whey powder. It can be assumed that this is related to the presence of a large number of bacteria in acid whey, which represent many species and strains [48]. Research has shown that the microflora of organic whey is dominated by lactic acid bacteria including *Lactobacillus* strains [48]. Our previous research indicated that the addition of acid whey caused the reduction of biogenic amines in fermented sausages, probably due to the presence of *Lactobacillus plantarum* in whey with a proven ability to produce amine oxidase enzymes and degrade biogenic amines [48]. In contrast to our findings, the research performed by Perea-Sanz et al. [48] showed that the reduction of nitrate addition to dry fermented sausages caused changes in the production of volatile compounds although nitrate reduction did not directly affect microbial growth but did affect microbial metabolism. The reduction of nitrate caused a decrease in volatile compounds derived from lipid oxidation and β-oxidation reactions.

5. Conclusions

The results of this study demonstrated that the amount of volatile compounds as well as the fatty acid profile of fermented sausages were determined by the type of meat and additives used. Beef sausages were characterized by a higher content of unsaturated fatty acids particularly susceptible to oxidation, a higher value of TBARS, and a higher amount of volatile compounds derived from lipid auto-oxidation. Generally, the elimination of nitrite from the composition of fermented sausages did not significantly affect the amount of volatile compounds, derived from lipid oxidation, bacterial metabolism, and others. However, an effect of the freeze-dried acid whey powder addition on the amount of some volatile components in uncured sausages, in particular those derived from bacterial metabolism, was observed. This may indicate a significant effect of the proportion of bacteria present in whey on their formation.

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