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ORIGINAL ARTICLE

Comparison of the effect of freeze-dried acid whey on physicochemical properties of organic fermented sausages made from beef and fallow deer meat

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Abstract The objective of this study was to compare the effect of freeze-dried acid whey on physicochemical properties and microbial changes of organic fermented sausages made from beef and fallow deer meat. Five formulations of sausages from each species were made. The results show that processing time and species of meat were the high significant factor on tested parameters. Variants and interactions between main factors influenced at different levels of significance on some tested attributes. At the end of processing fallow deer sausages were characterised by the lower pH (4.79 ± 0.01–4.90 ± 0.02 for fallow deer and 5.04 ± 0.00–5.25 ± 0.03 for beef sausages) and the content of 2-thiobarbituric acid reactive substances (1.54 ± 0.09–2.81 ± 0.23 and 1.64 ± 0.15–5.06 ± 0.25 respectively) than sausages made from beef meat. In conclusion, the addition of freeze-dried acid whey in varying amounts did not significantly affect the physicochemical characteristics of sausages from both fallow deer and beef. However, further research is needed to compare the effect of acid whey on the nutritional values of raw fermented sausages from fallow deer and beef.

Keywords Organic sausage · Acid whey · Physicochemical properties

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Introduction

Healthy lifestyle changes influenced the increase of consumer interest in organic food. Food from certified organic farming with high nutritious value, without preservatives or other unwanted additional substances are becoming more desired. For this reason, game meat or venison can also meet their expectations of consumers. Consumption of game meat (especially wild ruminants) can be beneficial for human health in conjunction to their lipid content and composition (Bureš et al. 2015; Maggiolino et al. 2019; Serrano et al. 2019a, b; Lorenzo et al. 2019). Moreover, sensory attributes of wild ruminants are scored higher than beef (Bureš et al. 2015). As Tomasevic et al. (2018) research has shown, consumers favour health benefits and nutritional properties of game meat, regarding it as more organic than other types of meat. Increasing availability of game meat and venison can lead to notice potential of this meat as an alternative to conventionally used red meat.

With the use of venison to produce fermented sausages, specific problems arise. During processing, chemical, biochemicals, physicochemical and microbiological changes are responsible for creating the typical properties and characteristic of fermented sausages (Lorenzo et al. 2012, 2014). Differences in raw materials, their composition and characteristic of endogenous microorganisms can significantly affects the changes during processing and nutrients composition of product (Chakanya et al. 2018; Zdanowska-Sąsiadek et al. 2018), thus comparison of technological parameters during production of organic fermented sausages made from beef and fallow deer meat seems justified.

An interesting way to enrich the nutritional value and prolong shelf life of fermented sausages without nitrite seems to be the addition of acid whey, by-product from

acid-coagulated dairy products processing. Acid whey is a rich source of lactic acid and microelements (Chandrapala et al. 2015). It can be also the source of many lactic acid bacteria species (Rzepkowska et al. 2017). Advantages of acid whey and its universal availability make that it finds more and more applications (De Giorgi et al. 2018; Ketterings et al. 2017). Use of acid whey and its positive impact on production is widely documented especially use of acid whey from organic cheese production (use of unpasteurised milk) allow to get fermented meat products without nitrites/nitrates characterised by good sensory quality and meeting the sanitary requirements. During production of dry cured meat acid whey had positive effects on the physico-chemical and sensory qualities, colour stability, or microbiological quality (Karwowska and Dolatowski 2017; Karwowska and Kononiuk 2018).

However, high concentration of lactic acid in acid whey is a main problem in storage that valuable source of protein. Presence of lactic acid make that producing whey powder or whey protein concentrate is difficult and thereat acid whey is usually discharged as effluent. Traditionally used process to produced whey is spray drying, which causes thermal degradation of many valuable substances present in organic acid whey (Saffari and Langrish 2014; Chandrapala et al. 2015; Chen et al. 2016). In this connection freeze-drying was proposed as an alternative method to preserve the organic acid whey which will additionally allow beneficial microorganisms survive. Therefore, the aim of this study was to compare the effect of freeze-dried acid whey on physicochemical properties (pH, water activity, colour parameters and chemical composition) and microbial changes of organic fermented sausages made from beef and fallow deer meat.

Materials and methods

Raw materials

The raw materials were beef meat, fallow deer meat, beef tallow and fallow deer tallow. The raw beef meat and tallow were collected from butcher Wasag (Bilgoraj, Poland) and came from organic certified breeding (PL-EKO-09/001/18). Fallow deer were collected directly after slaughter from organic breeding in Przytoczno (Poland). Fallow deer carcass was chilled for 48 h afterwards divided and trimmed.

For the production of sausages additives such as freeze-dried acid whey, sodium nitrate and sea salt were used. Liquid acid whey (pH 3.98 ± 0.03, moisture content $92.88 \pm 0.17\%$, lactic acid bacteria content 5.64 ± 0.18 log CFU g^{-1}) was obtained from traditional cottage production from certified, organic diary product plant (R. Janowski, Ludwinow, Poland, certificate no. PL-EKO-01-012678). Liquid acid whey after delivery was immediately frozen at − 50 °C and then freeze-dried using laboratory freeze drier (Labconco Free-Zone, USA). Acid whey powder was stored at − 50 °C until use. Before use acid whey was reconstituted through dissolution appropriate amount of acid whey powder in saline. Sodium nitrate was the chemical reagent (StanLab, Poland) and did not contain anti-caking agents. Sea salt was non-iodinated and without anti-caking agents (CurodiMare, Italy).

Sausages preparation

Two kinds of sausages were produced: beef and fallow deer. The sausages were made from meat and appropriate fat in a proportion of 90:10 (w/w). For each species of meat (beef and fallow deer) five formulations of fermented sausages were prepared: C (control sample with 2.8% addition of curing salt which contain 99.5% sea salt and 0.5% sodium nitrate), S (reference sample with 2.8% sea salt), SAW (tested sample with 2.8% sea salt and amount of acid whey powder corresponding to 5% liquid acid whey addition), SAW2 (tested sample with 2.8% sea salt and amount of acid whey powder corresponding to 10% liquid acid whey addition), SAW4 (tested sample with 2.8% sea salt and amount of acid whey powder corresponding to 20% liquid acid whey addition). 0.6% of glucose was added to all formulations. Water (5%) was added to control and reference samples, in case of tested sample exact amount of acid whey was dissolved in saline (amount was equivalent to the amount of water added to control and reference sample).

Refrigerated meats and fats were minced separately through a 10 mm grinding plate. Raw materials were divided into five batches for each species of meat and mixed with appropriate additives. The raw batters were stuffed into fibrous casings (diameter 65 mm). All sausages were weighted and hung in a temperature and humidity controlled chamber (16 °C, 80–90% relative humidity) for 20 days. During the ripening process all formulations from each meat were analysed. Parameters including pH, water activity, TBA-RS, total pigments content, haem iron content and colour parameters were measured at 0 (stuff), 10 and 20 day of ripening. The microbial content and composition of sausages (moisture, protein and fat content) were measured at the beginning (0 day) and at the end (20 day) of processing.

Analysis methods

pH value

Acidity of samples was measured in aqueous sample solution. The pH was measured with a digital pH meter CPC-501 (Elmetron, Zabrze, Poland) equipped with temperature sensor and pH electrode (ERH-111, Hydroment Gliwice, Poland). The pH of each sample was made in triplicate.

Water activity (a_w)

The water activity (a_w) of each samples was determined in triplicate at 20 °C using a LabMaster water activity meter (Novasina AG, Lachen, Switzerland).

TBA-RS determination

The content of 2-thiobarbituric acid reactive substances (TBA-RS) was used to analyse lipid oxidation. The measure was made six fold, according to the technique described by Pikul et al. (1989) using perchloric acid as a solvent. Results were expressed as equivalent of mg of malondialdehyde (MDA) per kilogram of sample.

Total pigments content and haem iron content

The total pigments and total haem iron content were determined according to procedure proposed by Hornsey (1956) with slight modification according to Karwowska and Dolatowski (2013). The measurements were carried out six times for each sample. The amount of total pigments and haem iron content were calculated according to Lee et al. (1999) and expressed in ppm.

Colour determination

Colour of sample was determined instrumentally, using X-Rite Colour Premiere 8200 spectrophotometer (X-Rite Inc., MI, USA). The measure were taken immediately after cutting the sample, using recommended by the Commission Internationale de l'Eclairage (CIE 2004) colour space L*a*b*. The measurement was carried out nine times for each sample.

Composition

The moisture, protein and fat content were determined in triplicate according to PN ISO 1442:2000, PN 75/A-04018, and PN ISO 1442:2000 respectively. Results of protein and fat were calculated as g per 100 g of dry matter.

Microbiological changes

Samples for microbiological analyses were taken after grinding and mixing the entire sample. All samples were taken in triplicate and carried out at Agrolab Group laboratory (Dęblin, Poland). The measurements of lactic acid bacteria (LAB) and *Enterobacteriaceae* were performed in accordance with PN ISO 15214:2002 and PN ISO 21528-2:2017-08 in appropriate order. All results were expressed in the colony forming unit per 1 g of product (CFU g^{-1}). Results were converted into log CFU g^{-1} and calculated as difference between content of bacteria in stuff (0 day) and in final product (20 day) (Δ).

Statistical analysis

The experiment included analysis of the influence of three main factors animal species (beef, fallow deer), additive used (curing salt, sea salt, freeze-dried acid whey in three different amounts) and processing time (0, 10, 20). The experiment was conducted in two independent replicates (batches). All quality parameters were measured in triplicate for each batch. The Statistica v.13 (Dell Inc, 2016) was used to the statistical develop of the results. Data was analysed using factorial ANOVA. Levene test was performed to confirm homogeneity of variance in groups. Relationships between subgroups have been specified based on post hoc Tukey's procedure. Differences were significant at $P \leq 0.05$.

Results and discussion

Table 1 shows statistical significance (P values) of the main factors and their interactions on the measured parameters. Day of processing (0, 10, 20) and species of meat (beef or deer fallow) highly significantly ($P < 0.0001$) determined all tested parameters. Type of formulations (variant) was significant factor ($0.0001 > P < 0.01$) for all parameters except for microbial changes (Δ LAB, Δ *Enterobacteriaceae*). All interactions between main factors affected highly significantly ($P < 0.0001$) on pH, water activity, TBA-RS, total pigments content and haem iron content of sausages.

Changes in physicochemical parameters

The pH values at the beginning of processing (0 day) were significantly higher in each samples from fallow deer meat compared to samples from beef (Table 2). After 10 days of processing the pH value was the lowest throughout the experiment which indicated fermentation phase of processing. According to others studies the lowest pH values

Table 1 Level of statistical significance (*P* value) influence of main factors and their interactions on the tested parameters

Attributes	Day (D)	Species of meat (S)	Variant (V)	D × S	D × V	S × V	D × S × V
Moisture content	****	****	****	****	*	***	ns
Protein content	****	****	****	****	ns	****	ns
Fat content	****	****	****	**	ns	****	*
Δ LAB	–	****	ns	–	–	ns	–
Δ *Enterobacteriaceae*	–	****	ns	–	–	ns	–
pH	****	****	***	****	****	****	****
Water activity	****	****	****	****	****	****	****
TBARS	****	****	****	****	****	****	****
Total pigments content	****	****	****	****	****	****	****
Haem iron content	****	****	****	****	****	****	****
L*	****	****	***	****	**	ns	***
a*	****	****	****	****	****	ns	****
b*	****	****	**	****	**	**	****

****$P \leq 0.0001$; ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; *ns* no statistical significance ($P > 0.05$)

Table 2 pH and water activity values of beef and fallow deer fermented sausages during processing (n = 3; mean ± SE)

	Day 0		Day 10		Day 20	
	Beef	Fallow deer	Beef	Fallow deer	Beef	Fallow deer
pH						
C	$5.64^{Ab} \pm 0.02$	$5.79^{Ba} \pm 0.01$	$5.04^{Ad} \pm 0.03$	$4.30^{ABf} \pm 0.02$	$5.25^{Ac} \pm 0.03$	$4.81^{ABe} \pm 0.05$
S	$5.63^{Ab} \pm 0.01$	$5.88^{Aa} \pm 0.04$	$4.94^{Bd} \pm 0.03$	$4.25^{Be} \pm 0.02$	$5.12^{Bc} \pm 0.02$	$4.90^{Ad} \pm 0.02$
SAW	$5.63^{Ab} \pm 0.01$	$5.80^{ABa} \pm 0.01$	$4.96^{ABc} \pm 0.03$	$4.34^{Ae} \pm 0.01$	$5.04^{Bc} \pm 0.00$	$4.85^{ABd} \pm 0.01$
SAW2	$5.56^{Ab} \pm 0.05$	$5.75^{BCa} \pm 0.02$	$5.01^{ABd} \pm 0.02$	$4.31^{ABf} \pm 0.03$	$5.21^{Ac} \pm 0.02$	$4.79^{Be} \pm 0.01$
SAW4	$5.56^{Ab} \pm 0.00$	$5.69^{Ca} \pm 0.02$	$5.00^{ABd} \pm 0.02$	$4.32^{ABf} \pm 0.02$	$5.21^{Ac} \pm 0.00$	$4.82^{ABe} \pm 0.02$
Water activity (a_w)						
C	$0.951^{Ab} \pm 0.001$	$0.977^{Aa} \pm 0.001$	$0.935^{Ac} \pm 0.001$	$0.938^{Ac} \pm 0.001$	$0.900^{Ae} \pm 0.002$	$0.914^{Ad} \pm 0.003$
S	$0.948^{Ab} \pm 0.003$	$0.979^{Aa} \pm 0.000$	$0.935^{Ac} \pm 0.002$	$0.941^{Abc} \pm 0.001$	$0.886^{Be} \pm 0.008$	$0.914^{Ad} \pm 0.002$
SAW	$0.950^{Aa} \pm 0.001$	$0.950^{Ba} \pm 0.009$	$0.935^{Ab} \pm 0.003$	$0.942^{Aab} \pm 0.001$	$0.883^{Bd} \pm 0.002$	$0.917^{Ac} \pm 0.002$
SAW2	$0.949^{Aa} \pm 0.003$	$0.951^{Ba} \pm 0.002$	$0.937^{Ab} \pm 0.003$	$0.938^{Ab} \pm 0.002$	$0.892^{ABd} \pm 0.001$	$0.910^{Ac} \pm 0.003$
SAW4	$0.949^{Aa} \pm 0.001$	$0.952^{Ba} \pm 0.001$	$0.937^{Ab} \pm 0.003$	$0.937^{Ab} \pm 0.001$	$0.887^{Bd} \pm 0.005$	$0.909^{Ac} \pm 0.006$

C sample with curing mixture, *S* sample with sea salt, *SAW* sample with sea salt and acid whey, *SAW2* sample with sea salt and double portion of acid whey, *SAW4* sample with sea salt and quadruple portion of acid whey

$^{A–C}$Means followed by the same letters in column are not significantly different at $P \leq 0.05$

$^{a–e}$Means followed by the same letters in row are not significantly different at $P \leq 0.05$

are observed during fermentation phase (Berardo et al. 2017). After 20 days pH values were slightly higher than after 10 days but also significantly lower than at the beginning. Similar relationships were also observed by Chakanya et al. (2018) during processing of salami made from game species and our other studies regarding the processing of beef sausages (Karwowska and Kononiuk 2018). The increase of pH value during processing may results from many factors including growth of yeasts or molds that produce substances affected on pH rise (Toldra 2012). From the other hand the same substances (ammonia, amines) can be released as a result of proteolytic reactions (protein breakdown, decarboxylation, deamination and other metabolisms of amino acids) and increase pH with the progress of proteolytic changes (Berardo et al. 2017). Despite higher pH values of fallow deer sausages at the beginning of processing, at 20 day sausages made from fallow deer meat was characterized by the significantly

lower value compared to beef sausages. It can indicate that acidifying substances formed more intensive in case of deer fallow meat. Significantly lower pH value after 23 days of salami processing made from fallow deer in comparison to pork was observed also by Chakanya et al. (2018). There were no significant effects of used additives on pH value although authors usually observed the influence of addition acid whey on drop in pH value due to the present of lactic acid (Karwowska et al. 2014; Karwowska and Kononiuk 2018).

The progressive changes of water activity through sausages processing are shown in Table 2. At the beginning of processing addition of acid whey significantly influenced the water activity values of sausages made from fallow deer (control and reference samples (C, S) were significantly higher than other variants). During processing in all types of sausages the a_w dropped. After 10 days of processing there were no significant difference between the same variants of sausages from different species meat. At the end of processing the sausages made from beef meat were characterized by the lower water activity than sausages from fallow deer. Lower water activity in case of sausages made from beef is a result of difference in moisture content. Sausages made from fallow deer at the end of processing were characterized by higher content of water than beef sausages (Table 6). Nevertheless water activity of all samples at the end of process were below 0.920 which is described as minimal value required for growth of many unwanted microorganisms including *Listeria monocytogenes* (USDA 2011).

Changes in TBA-RS value during processing of fermented sausages are shown in Table 3. The results obtained showed much higher TBA-RS value in comparison to those presented by other authors (Chakanya et al. 2018; Karwowska and Dolatowski 2017) and similar to those obtained by Karwowska and Dolatowski (2017). These differences may result from the assay method. Recently Zhang et al. (2019) used two extraction methods to measure TBA-RS and obtained different results. Therefore, comparing TBA-RS results and consumer acceptance threshold with other authors is difficult and focusing on analysis of obtained results only. Through the processing of sausages, TBA-RS of all samples increased. The highest differences (between TBA-RS value at the beginning and at the end of processing) were observed in case of sausages from beef, especially reference sample (S). Changes in TBA-RS values between sausages made from different meat species may result of different meat tallow used (suitable for the meat used) which are characterised by different fatty acid composition (Bureš et al. 2015) and different susceptibility to oxidation. As expected the lowest TBA-RS values were observed in control samples with curing salt addition (C) irrespective of processing stages and used meat species, which confirmed antioxidant effect of nitrite (Honikel 2008). At every stages sample with acid whey addition (mainly double and quadruple portion) were characterized by higher TBA-RS value than control (C) or reference (S) samples. This indicated that addition of acid whey do not protects lipid from oxidation, similar trends were observed by Karwowska and Kononiuk (2018).

The highest content of total pigments and haem iron in samples were observed at the end of processing, and was similar in both meat species used (Table 3). The main reason of that was the drop in water content and compaction of ingredients (Table 6). Our results show that haem iron in deer fallow do not significantly different from beef. Higher content of total pigments and haem iron in samples with curing mixture addition after 10 and 20 days indicated preventing effect of nitrates on pigments degradation during processing in both types of sausages. Nevertheless changes in pigments and haem iron content are correlated with changes in TBA-RS value (Pearson correlation coefficient are $R = 0.481$ and $R = 0.474$ respectively). Presence of haem iron may cause prooxidative effect, thus increase in total pigments and haem iron content affect on intensively of oxidative changes and thus TBA-RS value is increased (Carlsen et al. 2005). In contrast to Okabe et al. (2002) and popular opinion (Chakanya et al. 2018) that haem iron content in game meat are higher and enhanced lipid oxidation, our study shows that despite the content of haem iron in sausages made from beef and fallow deer were not significantly different, the number of lipid oxidation products (TBA-RS) were statistically different in both meat species.

Changes in colour parameters during the processing of sausages were shown in Table 4. Through processing the highest lightness (L*) value of each sample were observed at 10 days of processing. This was expected due to the lowest pH value in this stage. Correlation between these parameters (Pearson correlation coefficient was − 0.579) is the result of changes in the redox form of pigments caused by acidity of sample.

The samples from fallow deer meat were characterized by the higher value of redness (a*) compared to corresponding variants of beef samples (Table 4). Due to similar contents of total pigments of beef and fallow deer sausages (Table 3), differences in redness indicated the changes in redox form of myoglobin. Moreover, fallow deer meat is usually characterized by lower colour parameters compared to beef meat (Bureš et al. 2015; Farouk et al. 2007). After 10 days of processing a* parameter values significantly increased in case of each sausages made from beef and decreased in case of sausages made from fallow deer (except control sample (C) with curing mixture addition). These dependencies may arise from the species-specific

Table 3 TBA-RS value, total pigments content and haem iron content of beef and fallow deer sausages during processing (n = 6; mean ± SE)

	Day 0		Day 10		Day 20	
	Beef	Fallow deer	Beef	Fallow deer	Beef	Fallow deer
TBA-RS value (equivalent of mg MDA kg of sample^{-1})						
C	$1.10^{Ac} \pm 0.22$	$1.26^{Bbc} \pm 0.09$	$1.41^{BCabc} \pm 0.17$	$1.39^{Cabc} \pm 0.06$	$1.64^{Da} \pm 0.15$	$1.54^{Cab} \pm 0.09$
S	$1.07^{Ac} \pm 0.14$	$1.40^{ABcd} \pm 0.12$	$1.29^{Ccd} \pm 0.09$	$1.51^{BCc} \pm 0.15$	$5.06^{Aa} \pm 0.25$	$1.99^{Bb} \pm 0.12$
SAW	$1.16^{Ad} \pm 0.23$	$1.67^{Abc} \pm 0.14$	$1.35^{Ccd} \pm 0.10$	$1.67^{BCbc} \pm 0.08$	$2.46^{Ca} \pm 0.16$	$1.99^{Bb} \pm 0.14$
SAW2	$1.06^{Ad} \pm 0.15$	$1.45^{ABc} \pm 0.07$	$1.69^{ABc} \pm 0.10$	$1.78^{Bc} \pm 0.12$	$2.84^{Ba} \pm 0.22$	$2.25^{Bb} \pm 0.10$
SAW4	$1.12^{Ad} \pm 0.14$	$1.44^{ABd} \pm 0.28$	$1.80^{Ac} \pm 0.25$	$2.23^{Ab} \pm 0.05$	$2.96^{Ba} \pm 0.12$	$2.81^{Aa} \pm 0.23$
Total pigments content (ppm)						
C	$159.57^{Ad} \pm 2.97$	$157.42^{Ad} \pm 13.88$	$208.53^{Ac} \pm 14.85$	$232.67^{Ab} \pm 15.13$	$271.21^{Aa} \pm 6.41$	$276.08^{Aa} \pm 7.15$
S	$158.67^{Ac} \pm 3.49$	$136.57^{Bd} \pm 4.82$	$200.15^{ABb} \pm 6.50$	$192.89^{Bb} \pm 3.72$	$228.93^{Ba} \pm 16.84$	$231.20^{Ba} \pm 5.14$
SAW	$154.13^{Ac} \pm 3.03$	$140.19^{ABc} \pm 22.99$	$192.21^{ABb} \pm 4.52$	$189.38^{Bb} \pm 3.59$	$236.30^{Ba} \pm 5.07$	$222.59^{BCa} \pm 10.66$
SAW2	$162.86^{Ad} \pm 2.78$	$150.39^{ABd} \pm 6.71$	$184.96^{Bc} \pm 4.87$	$185.75^{Bc} \pm 2.90$	$279.82^{Aa} \pm 7.05$	$211.82^{BCb} \pm 2.31$
SAW4	$154.02^{Ad} \pm 2.42$	$141.33^{ABd} \pm 4.44$	$182.01^{Bc} \pm 6.39$	$189.04^{Bc} \pm 4.28$	$248.31^{Ba} \pm 19.74$	$210.69^{Cb} \pm 7.32$
Haem iron content (ppm)						
C	$14.07^{Ad} \pm 0.26$	$13.88^{Ad} \pm 1.22$	$18.39^{Ac} \pm 1.31$	$20.52^{Ab} \pm 1.33$	$23.92^{Aa} \pm 0.57$	$24.35^{Aa} \pm 0.59$
S	$13.99^{Ac} \pm 0.31$	$12.05^{Bd} \pm 0.42$	$17.65^{ABb} \pm 0.57$	$17.01^{Bb} \pm 0.33$	$20.19^{Ba} \pm 1.49$	$20.39^{Ba} \pm 0.45$
SAW	$13.59^{Ac} \pm 0.27$	$12.37^{ABc} \pm 2.03$	$16.95^{ABb} \pm 0.40$	$16.70^{Bb} \pm 0.32$	$20.84^{Ba} \pm 0.45$	$19.63^{BCa} \pm 0.94$
SAW2	$14.36^{Ad} \pm 0.25$	$13.26^{ABd} \pm 0.59$	$16.31^{Bc} \pm 0.43$	$16.38^{Bc} \pm 0.26$	$24.68^{Aa} \pm 0.62$	$18.68^{BCb} \pm 0.20$
SAW4	$13.58^{Ad} \pm 0.21$	$12.47^{ABd} \pm 0.39$	$16.05^{Bc} \pm 0.56$	$16.67^{Bc} \pm 0.38$	$21.90^{Ba} \pm 1.74$	$18.58^{Cb} \pm 0.65$

C sample with curing mixture, *S* sample with sea salt, *SAW* sample with sea salt and acid whey, *SAW2* sample with sea salt and double portion of acid whey, *SAW4* sample with sea salt and quadruple portion of acid whey

$^{A–C}$Means followed by the same letters in column are not significantly different at $P \leq 0.05$

$^{a–c}$Means followed by the same letters in row are not significantly different at $P \leq 0.05$

differences in colour phenomena (Farouk et al. 2007). At the end of processing beef sausage samples with different amounts of acid whey (SAW, SAW2, SAW4) were characterized by higher redness than reference sample (S). As far as sausages made from fallow deer are concerned, there were no significant difference observed between samples S, SAW, SAW2 and SAW4, however sample SAW4 was characterized by the higher value of a* parameter. Differences in changes of a* parameters during processing between beef and fallow deer sausage, despite similar content of total pigments on each stages indicated the biochemical reason of that changes. According to Bozkurt and Bayram (2006) during production of dry cured meat at first days nitrogenous compounds from meat react with myoglobin and formed desired colour pigment (increase of a* parameter is observed), then fallows denaturation of formed pigments and a* parameters decreased. In our study similar trend was observed in case of beef meat. Changes in fallow deer samples points to the same with the exception that denaturation changes of pigments may take place earlier according to the species-specific differences like different endogenous enzyme activity (Suman et al. 2014; Bozkurt and Bayram 2006; Bureš et al. 2015).

During production yellowness of samples was significantly decreased (Table 4). Results of stuff samples (0 day) indicated that samples made from fallow deer were characterized by significantly higher yellowness than samples from beef meat. Statistical analysis displayed that yellowness was not significantly influenced by the additives used, except the last stage of sausages from fallow deer processing. The exception was the results obtained on the 20th day of production for samples from fallow deer. The samples with the addition of acid whey were characterized by significantly higher yellowness than control or reference samples, especially double (SAW2) and quadruple (SAW4) portion of acid whey influenced this parameter. Changes in yellowness during processing of dry cured meat were explained by Pérez-Alvarez et al. (1999) as the result of oxygen consumption by microorganisms and hence decrease in oxymyoglobin content.

Microbiological changes

Microbiological changes during processing of fermented meat are very important as the growth of some bacteria as lactic acid bacteria is desired and determines the correct

Table 4 Colour parameters L*, a*, b* value during processing of fermented sausages (n = 9; mean ± SE)

	Day 0		Day 10		Day 20	
	Beef	Fallow deer	Beef	Fallow deer	Beef	Fallow deer
*L*parameter*						
C	$41.90^{Ac} \pm 1.60$	$46.69^{Aab} \pm 2.77$	$48.28^{Aa} \pm 2.55$	$49.76^{Aa} \pm 2.29$	$43.09^{Bbc} \pm 3.13$	$50.33^{Aa} \pm 0.79$
S	$43.01^{Ab} \pm 1.84$	$49.16^{Aa} \pm 5.84$	$48.77^{Aa} \pm 0.75$	$52.29^{Aa} \pm 2.09$	$49.61^{Aa} \pm 3.54$	$49.35^{ABCa} \pm 2.69$
SAW	$42.21^{Ab} \pm 2.18$	$49.14^{Aa} \pm 2.44$	$49.20^{Aa} \pm 1.18$	$51.74^{Aa} \pm 2.52$	$47.68^{Aa} \pm 1.53$	$50.15^{ABa} \pm 1.11$
SAW2	$40.86^{Ad} \pm 0.62$	$47.34^{Abc} \pm 2.35$	$50.48^{Aab} \pm 1.90$	$52.65^{Aa} \pm 2.06$	$46.12^{ABc} \pm 2.13$	$46.10^{BCc} \pm 1.45$
SAW4	$41.67^{Ac} \pm 1.74$	$47.00^{Aab} \pm 1.83$	$48.79^{Aab} \pm 2.39$	$50.88^{Aa} \pm 1.87$	$46.55^{ABb} \pm 2.64$	$45.93^{Cb} \pm 1.31$
a parameter*						
C	$6.42^{Ad} \pm 0.40$	$13.82^{Aab} \pm 2.08$	$13.14^{Aabc} \pm 0.98$	$14.30^{Aa} \pm 1.70$	$11.80^{Ac} \pm 0.56$	$12.39^{Ac} \pm 0.40$
S	$6.03^{Ac} \pm 0.56$	$12.05^{Ba} \pm 1.90$	$10.03^{Bb} \pm 0.42$	$9.62^{Bb} \pm 0.94$	$2.63^{Ce} \pm 0.35$	$4.91^{Bd} \pm 0.45$
SAW	$6.02^{Ac} \pm 0.37$	$13.50^{ABa} \pm 1.69$	$9.80^{Bb} \pm 0.58$	$10.69^{Bb} \pm 0.45$	$4.39^{Bc} \pm 1.43$	$4.68^{Bc} \pm 0.28$
SAW2	$6.91^{Ac} \pm 0.61$	$12.99^{ABa} \pm 1.23$	$10.05^{Bb} \pm 0.59$	$10.08^{Bb} \pm 0.80$	$3.46^{BCc} \pm 0.63$	$5.91^{Bc} \pm 0.42$
SAW4	$5.96^{Ac} \pm 0.28$	$13.48^{ABa} \pm 1.53$	$10.33^{Bb} \pm 0.79$	$10.18^{Bb} \pm 1.44$	$3.71^{BCc} \pm 0.86$	$6.13^{Bc} \pm 0.46$
b parameter*						
C	$10.45^{Aab} \pm 1.61$	$12.20^{Aa} \pm 1.52$	$7.70^{Acd} \pm 0.58$	$8.41^{Abc} \pm 1.10$	$6.12^{Ad} \pm 0.53$	$7.04^{Ccd} \pm 0.40$
S	$9.17^{Ab} \pm 2.05$	$12.26^{Aa} \pm 2.15$	$8.48^{Abc} \pm 0.64$	$8.33^{Abc} \pm 0.87$	$6.47^{Ac} \pm 0.63$	$7.38^{Cbc} \pm 0.25$
SAW	$10.93^{Ab} \pm 3.63$	$13.60^{Aa} \pm 0.97$	$8.32^{Ac} \pm 0.38$	$8.51^{Ac} \pm 1.11$	$6.08^{Ad} \pm 0.45$	$8.51^{BCc} \pm 0.52$
SAW2	$9.75^{Abc} \pm 0.91$	$11.90^{Aa} \pm 1.53$	$8.52^{Ac} \pm 0.57$	$8.41^{Ac} \pm 0.86$	$4.71^{Ad} \pm 0.34$	$10.84^{Aab} \pm 0.52$
SAW4	$9.50^{Abc} \pm 0.68$	$12.65^{Aa} \pm 1.62$	$9.62^{Abc} \pm 0.93$	$8.05^{Acd} \pm 1.23$	$5.96^{Acd} \pm 0.40$	$10.57^{ABab} \pm 0.34$

C sample with curing mixture, *S* sample with sea salt, *SAW* sample with sea salt and acid whey, *SAW2* sample with sea salt and double portion of acid whey, *SAW4* sample with sea salt and quadruple portion of acid whey

[A–C]Means followed by the same letters in column are not significantly different at $P \leq 0.05$

[a–c]Means followed by the same letters in row are not significantly different at $P \leq 0.05$

Table 5 Differences in count of lactic acid bacteria (LAB) and *Enterobacteriaceae* between the beginning and end of processing (n = 3; mean ± SE)

	Δ LAB (log CFU g^{-1})		Δ *Enterobacteriaceae* (log CFU g^{-1})	
	Beef	Fallow deer	Beef	Fallow deer
C	$2.01^{abc} \pm 0.20$	$0.82^{cd} \pm 0.17$	$-1.92^{c} \pm 0.79$	$-3.28^{abc} \pm 0.24$
S	$2.15^{ab} \pm 0.49$	$0.81^{cd} \pm 0.13$	$-2.34^{abc} \pm 0.31$	$-3.32^{ab} \pm 0.09$
SAW	$2.39^{a} \pm 0.18$	$0.41^{d} \pm 0.15$	$-1.97^{bc} \pm 0.38$	$-3.15^{abc} \pm 0.16$
SAW2	$2.41^{a} \pm 0.72$	$0.89^{bcd} \pm 0.08$	$-2.57^{abc} \pm 0.35$	$-3.51^{a} \pm 0.05$
SAW4	$2.06^{abc} \pm 0.24$	$0.81^{cd} \pm 0.18$	$-2.64^{abc} \pm 0.11$	$-3.26^{abc} \pm 0.40$

C sample with curing mixture, *S* sample with sea salt, *SAW* sample with sea salt and acid whey, *SAW2* sample with sea salt and double portion of acid whey, *SAW4* sample with sea salt and quadruple portion of acid whey

[a–d]Means with the same letter are not significantly different at $P \leq 0.05$

course of the fermentation process. From the other hand presence of pathogenic microorganisms adversely affects the food safety of products. Changes in lactic acid bacteria (LAB) and *Enterobacteriaceae* count of experimental samples were shown in Table 5. During processing, significantly higher increase of lactic acid bacteria in sausages samples from beef compared to fallow deer meat was observed.

Increase of lactic acid bacteria count during processing of fermented meat is very important as mainly lactic acid bacteria produce lactic acid and bacteriocins, which could be used as an effective natural preservative for meat (Woraprayote et al. 2016). Despite this the count of *Enterobacteriaceae* was lower in case of sausages from fallow deer. It could mean that increase of lactic acid bacteria count are not effective determinant of the decrease in *Enterobacteriaceae* count. Analysis the initial and final

Table 6 Protein and fat content of sausages made from fallow deer and beef meat (n = 3; mean ± SE)

Parameter	Variant	Type of meat	
		Beef	Fallow deer
Protein (g/100 g of dry matter)	C	$61.97^{c} \pm 0.45$	$66.71^{a} \pm 0.27$
	S	$56.28^{f} \pm 0.23$	$67.09^{a} \pm 0.37$
	SAW	$59.77^{d} \pm 0.39$	$66.91^{a} \pm 0.38$
	SAW2	$58.61^{de} \pm 0.32$	$64.80^{b} \pm 0.44$
	SAW4	$57.21^{ef} \pm 0.26$	$64.49^{b} \pm 0.40$
Fat (g/100 g of dry matter)	C	$37.87^{d} \pm 0.20$	$33.44^{f} \pm 0.55$
	S	$43.58^{a} \pm 0.20$	$32.97^{f} \pm 0.13$
	SAW	$40.49^{c} \pm 0.28$	$33.45^{f} \pm 0.41$
	SAW2	$41.92^{b} \pm 0.18$	$35.40^{e} \pm 0.15$
	SAW4	$42.49^{ab} \pm 0.24$	$35.41^{e} \pm 0.19$

C sample with curing mixture, *S* sample with sea salt, *SAW* sample with sea salt and acid whey, *SAW2* sample with sea salt and double portion of acid whey, *SAW4* sample with sea salt and quadruple portion of acid whey

[a–f]Means followed by the same letters are not significantly different at $P \leq 0.05$

amount of these bacteria could be more accurate. Correlation between LAB and *Enterobacteriaceae* content during processing of dry cured products is widely reported by other authors (Greppi et al. 2015). One of the reasons of this relationship can be bacteriocins produced by lactic acid bacteria. Woraprayote et al. (2016) studied bacteriocins from many species of lactic acid bacteria and showed their broad antimicrobial spectrum against pathogenic microorganisms including, among others *Enterobacteriaceae* or *Listeria monocytogenes*. No difference between variants despite the adding in some cases additional LAB (SAW, SAW2, SAW4) indicated that amount of medium for bacteria can be insufficient and limiting their growth. The low water activity value (Table 2) after 20 days of ripening could also have the limiting effect.

Changes in chemical composition

The composition of sausages were shown in Table 6. The higher difference in composition was observed between sausages made from different type of meat. Sausages from deer were characterised by significantly higher content of protein and lower content of fat. Difference in protein content range between 7.6% for samples with curing mixture addition (C) to 19.2% for samples with sea salt addition (S). Sausages made from beef had from 13.2% (sample C) to 30% (sample S) more fat than corresponding fallow deer sausage variants.. That results correspond with results of other authors and confirmed that farmed fallow deer muscles are characterized by lower fat content than beef (Ludwiczak et al. 2017; Bureš et al. 2015). However studies of many authors (Bureš et al. 2015; Chakanya et al. 2018; Ludwiczak et al. 2017) showed that comparison of protein content in beef and fallow deer muscle is difficult due to its dependence on many conditions including sex, breeding, production system and many others.

Conclusion

The result of present study shows various changes occurring during processing of organic sausages made from two meat species (beef and fallow deer). After 20 days of processing sausages made from fallow deer were characterised by higher acidity and water activity, lower level of lipid oxidation and favourable colour parameters. The increase in the bacteria content was greater in case of beef sausages despite this decrease in pathogenic microorganisms were greater in fallow deer sausages.

The addition of freeze-dried acid whey in varying amounts did not significantly affect the physicochemical characteristics of sausages from both fallow deer and beef. Further research is needed to compare the effect of acid whey on the nutritional values of raw fermented sausages from fallow deer and beef.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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