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EVOLUTION OF FREE AMINO ACIDS, BIOGENIC AMINES AND *N*-NITROSOAMINES THROUGHOUT AGEING IN ORGANIC FERMENTED BEEF*

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ABSTRACT

Background. In recent years, interest in uncured meat products has grown and studies were carried out on the use of substances which could replace nitrites, such as acid whey. In spite of this problem in fermented meat products, there is no information regarding the effects of prolonged ageing on the formation of chemical (nitrosoamines, biogenic amines, secondary lipid oxidation products) and microbiological (*L. monocytogenes*, *S. aureus, OLD*) toxicants in fermented beef marinated with acid whey. The aim of this study was to determine the selected pathogenic bacteria, biogenic amines, *N*-nitrosamines contents in fermented beef subjected to extended ageing.

Material and methods. In this study, selected pathogenic bacteria, *N*-nitrosamines, biogenic amines, amino acids, TBARS values changes during the ageing of fermented beef marinated with acid whey were analyzed in 0-, 2- and 36-month-old samples.

Results. The pH values of fermented beef aged for 2 months (5.68, 5.49 and 5.68 respectively) were significantly lower ($p < 0.05$) than those obtained after the end of the manufacturing ripening period (5.96, 5.97 and 5.74 respectively), which confirmed the effectiveness of the fermentation process of acidification on beef. The high Lactic Acid Bacteria content (5.64–6.30 log cfu/g) confirmed this finding. Histamine was not detected in either of the products. The highest concentration of total biogenic amine (i.e. 1159.0 mg/kg) was found in fermented beef marinated with acid whey, whereas a total of only 209.8 mg/kg, was observed in control beef with nitrate and nitrite. *N*-nitrosamines were not detected in any of the ageing beef samples.

Conclusion. In this study, marinating beef in acid whey did not inhibit the production of biogenic amines in the samples analyzed. The high concentration of FAAs, the potential precursor of BA, could lead to intense peptidase activity. The results obtained indicate that biogenic amines are not direct precursors for nitrosamines formation in fermented beef. The LAB strain from acid whey reduced the pH value during the first stages of ageing and ensured the microbiological safety of the product not only in the first stage of fermentation but also at the end of ageing (36 months).

Key words: acid whey, fermentation, biogenic amine, oxidation, nitrosoamine, free amino acids

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INTRODUCTION

The preservation of meat by microorganism fermentation has been used for thousands of years. During this process, numerous substances form in the products, such as organic acids, alcohols, aldehydes, ketones, which have an influence on the quality and storage stability of the product. Nitrite (KNO_2/E249 and $NaNO_2$/E250) and nitrate ($NaNO_3$/E251 and KNO_3/E252) are generally used to avoid the growth of putrefactive and pathogenic bacteria, like *Clostridium botulinum* in fermented meat products. Moreover, nitrite promotes color formation, delays PUFA oxidation and gives the product a typical cured meat odour and taste. In addition to this benefits, nitrite can be converted into the nitrosating agent nitric oxide (NO), which can react with secondary amines to form *N*-nitrosamines (Honikel, 2008). The group of nitrosamines includes volatile (VNA) and non volatile nitrosamines (NVNA). The majority of the VNAs are known to be carcinogenic. According to the classification of carcinogenic compounds, *N*-nitrosodimethylamine (NDMA), and *N*-nitrosodiethylamine (NDEA) belong to the group of the probable carcinogens, whereas *N*-nitrosodibutylamine (NDBA), *N*-nitrosopiperidine (NPIP) and *N*-nitrosopyrrolidine (NPYR) belong to the group of the possible carcinogens (Yurchenko and Mölder, 2005). What is more, Karovičová and Kohajdová (2005) claim that putrescine (PUT) from ornithine, cadaverine (CAD) from lysine, spermine (SPM) and spermidine (SPD) from putrescine can be the precursors of carcinogenic *N*-nitrosamines. For example, CAD can be converted into *N*-nitrosopiperidine (NPIP) and PUT, SPM, SPD can form *N*-nitrosopyrrolidine (NPYR). It has also been suggested that NPIP can be formed from cadaverine (Wei et al., 2009). BA are organic bases of a low molecular weight with an aliphatic, aromatic or heterocyclic structure formed through the decarboxylation of free amino acids or by amination and transamination of aldehydes and ketones (Jansen et al., 2003). Fermented meat products have been reported to contain BA (Galgano et al., 2009; Ruiz-Capillas and Jiménez-Colmenero, 2004). The concentration of BA could be influenced by the enzymatic activity of microbiota or the level of lipid oxidation in fermented meat products. Biogenic amines may induce migraines and hypertensive crises, fever, nausea, urticaria in sensitive individuals (De Mey et al., 2014; Latorre-Moratella et al., 2008). The EFSA has established that a maximum daily intake of 50 mg of histamine, and 600 mg of tyramine can be considered safe for healthy individuals (EFSA, 2011). The presence of putrescine and cadaverine can enhance the toxicity of histamine and tyramine. In view of food safety, concentrations of biogenic amines and *N*-nitrosamine must be avoided. Moreover, fermented meat products constitute one of the foods in which high quantities of toxic compounds can be found as a consequence of the use of poor-quality raw materials, microbial contamination and inadequate conditions during processing and storage. Toxicants borne in fermented meat during processing and storage, such as pathogenic microorganism and botulinum toxin, residual nitrate and nitrite, nitrosamines, and during peroxidation are products of polyunsaturated fatty acids.

The primary oxidation products of PUFAs (hydroperoxides, epoxides etc.) decompose further into toxic secondary oxidation products (aldehydes, ketones and alkanes etc.) possessing the rancid smell of spoiled matter. These compounds may break the transmission of a nervous impulse and produce DNA-adducts expressing mutagenicity and carcinogenicity, leading to the increase in the probability of cancer and atherosclerosis developing (Püssa, 2013). Microbial hazards are related to the presence of pathogens in meat products. It has been concluded that *Clostridium*, *L. monocytogenes*, *St. aureus* and *Salmonella* species are the most serious risk for consumers connected with uncured fermented meat products consumption.

The development of technologies to obtain organic meat products free or nearly free from toxicants is a challenge for the meat industry. In recent years, interest in uncured meat products has grown and studies were carried out on the use of substances which could replace nitrite, for example acid whey (Wójciak et al., 2014; Wójciak et al., 2015). In spite of this problem in fermented meat products, there is no information regarding the effects of prolonged ageing on the formation of chemical (nitrosoamines, biogenic amines, secondary lipid oxidation products) and microbiological (*L. monocytogenes*, *S. aureus, OLD*) toxicants in fermented beef marinated with acid whey.

The aim of this work, which forms part of a wider study dedicated to the possibility of eliminating nitrate

and nitrite addition from non-heated and heated meat products, was to determine the selected pathogenic bacteria, biogenic amines, *N*-nitrosamine, and PUFA oxidation products of fermented beef subjected to extended ageing. In this study, pH, a_w, ORP, and TBARS value changes during the process of ageing fermented beef marinated with acid whey were analyzed in 0, 2 and 36-month-old samples. Selected pathogenic bacteria and free amino acids were measured after ripening and at the end of a prolonged storage period. *N*--nitrosamines and biogenic amine were analyzed only in 36-month-old samples.

MATERIAL AND METHODS

Experimental procedures

The raw material for the production of fermented beef was cooled (24 h, 2–4°C). Semimembranous muscle (Lat. *musculus semimembranosus)* was obtained from Limousin cattle with a body weight of approx. 400–450 kg, which were bred in the organic farming system. Acid whey was obtained from a local farm, which organically breeds dairy cattle and is a producer of organic dairy products. The *semimembranous* muscles were divided into three groups. The first group of *semimembranous* muscle was marinated in acid whey for 24 hours at a temperature of 4°C. Afterwards, sea salt was added to the meat. The quantity was 3.0% in relation to the meat mass. The second group of *semimembranous* muscle was cured (3.0%) and the third group was salted (3.0%). These products were subjected to a 21-day ripening process at a temperature of 16°C and a relative humidity of 75–85%. After ripening, the beef was cold-smoked (approx. 26°C) and was subsequently vacuum-packed and stored at a temperature of 4°C. The products were tested after the ripening process (21 days) and after extended ageing (2 and 36 months). The following experimental variants were prepared:

- S – control ripening beef with the 3% addition of sea salt
- N – control ripening beef with the 3% addition of the curing mixture
- W – ripening beef marinated in acid whey for 48 h, with the 3% addition of sea salt.

Analysis of samples

pH value and water activity deterioration. Acidity was determined by measuring the pH value with the use of a CPC 501 digital pH/conductivity meter (Elmetron, Zabrze, Poland) and ERH-111-type combined electrode (Hydromet, Gliwice, Poland). The analysis of the water activity (a_w) in the product was conducted at a temperature of 20°C with the use of a LabMaster device (Novasina AG, Lachen, Switzerland).

Oxidation-reduction potential. Oxidation-reduction potential was determined with the use of an ERPt--13-type platinum combined electrode with the use of a digital CPC-501 pH conductivity meter (Elmetron, Zabrze, Poland) on the basis of the methods described by Ahn and Nam (2003).

TBARS value. TBARS was determined on the basis of methods described by Pikul et al. (1989). The intensity of the pink colour created due to the reaction of fat oxygenation products with 2-thiobarbituric acid was measured with the use of a Nikole Evolution 300 spectrophotometer (Thermo Elektron Corporation) at a wavelength of 532 nm.

Microbial analysis. LAB was assayed according to PN ISO 15214:2002 and selected pathogenic bacteria were also assayed. Assaying of *S. aureus* was performed on the basis of PN ISO 6888-2:2001/A1:2004, whereas *L. monocytogenes* was performed on the basis of the Rapid Lmono method developed by Biorad (QMP-504-EC-27-51-1). Microbial tests to determine total viable counts were performed according to ISO 4833:2004. The results were reported as colony-forming units per gram of the product [cfu/g].

Determination of dry matter. Dry matter was determined by air drying the beef sample at 105°C until it reached a constant weight.

Free amino acid concentration. Free amino acid content was determined using the automatic amino acid analyzer AAA 400 (Ingos Ltd. Czech Republic) equipped with an Ostion LG ANB ion-exchange column (36 × 0.37 cm) kept at 70°C according to the method described by Stadnik and Dolatowski (2015). Determination of free amino acids (FAAs) was

confirmed by comparing the retention times and peak areas of the particular amino acid standards with those of the components present in the samples. FAA concentration was expressed as mg/kg of dry matter (DM).

Biogenic amines content. The contents of BA were determined using an automatic amino acid analyzer AAA 400 (Ingos Ltd. Czech Republik) equipped with an Ostion LG ANB ion-exchange column (11.5×0.45 cm) kept at 76°C, according to the method described by Stadnik and Dolatowski (2015). Determination of BA was confirmed by comparing the retention times and peak areas of the particular BA standards with those of the components present in the samples. BA content was expressed as mg/kg of meat.

***N*-nitrosamines.** Volatile nitrosamines were extracted by low-temperature vacuum distillation according to the method recommended by FSIS in Agrolab Group (Germany). Volatile nitrosamines had been subjected to the addition of water, sodium hydroxide and glycerin, as well as polyethylene glycol steam distillation separated from the sample by a vacuum. The aqueous distillate is either alkalized over a silica gel column with dichloromethane extracted after acidification or shaken in a separating 'jug' with the same solvent. The concentrated extracts were analyzed with a gas chromatograph with chemiluminescence detector quantitative.

Statistical analysis. Two batches of fermented beef were prepared and all specific experiments were repeated at least two times. The data reported were analyzed by two-way analysis of variance (ANOVA) at $p \leq 0.05$. The significance of the differences between treatments at the same storage time and the same treatments at different storage times was determined (at the significant level $p \leq 0.05$) using the T-Tukey's range test.

RESULTS AND DISCUSSION

The pH values of fermented beef aged for 2 months (5.68, 5.49 and 5.68 respectively) were significantly lower ($p < 0.05$) than those obtained after the end of manufacturing ripening period (5.96, 5.97 and 5.74 respectively), which confirmed the effectiveness of the fermentation process of acidification on beef. The high LAB content confirmed this finding (Table 4). Similar

Table 1. pH value, water activity, oxidation-reduction potential and TBARS value in fermented beef examined during ageing period (36 months)

Sample	Time month	pH	a_w	ORP mV	TBARS mg/kg
S	0	5.96 ±0.03^{aA}	0.938 ±0.01^{aA}	306.43 ±4.87^{abA}	4.03 ±0.22^{aA}
	2	5.68 ±0.01^{aA}	0.920 ±0.01^{aB}	341.32 ±1.12^{aB}	4.32 ±0.03^{aB}
	36	6.16 ±0.03^{bA}	0.908 ±0.01^{bC}	356.17 ±4.42^{aC}	1.20 ±0.09^{aC}
N	0	5.97 ±0.03^{aB}	0.938 ±0.01^{bA}	309.10 ±4.57^{bA}	3.41 ±0.11^{bA}
	2	5.49 ±0.01^{bB}	0.937 ±0.01^{aB}	338.15 ±1.61^{bB}	2.20 ±0.07^{bB}
	36	6.08 ±0.01^{aB}	0.901 ±0.01^{aC}	330.29 ±1.71^{bC}	0.61 ±0.08^{cC}
W	0	5.74 ±0.03^{aC}	0.934 ±0.01^{bA}	300.73 ±1.54^{aA}	3.49 ±0.09^{bA}
	2	5.68 ±0.01^{bC}	0.922 ±0.01^{cB}	325.08 ±2.92^{cB}	2.40 ±0.07^{bB}
	36	6.09 ±0.01^{aC}	0.901 ±0.02^{aC}	340.85 ±2.66^{cC}	1.19 ±0.12^{aC}

Mean value ±standard error.
Samples: S – control with sea salt (3%), N – control with curing mixture (3%), W – experimental with acid whey and sea salt (3%).
Means followed by the same letters within columns (capital letters) and rows (lower case letters) are not significantly different at $p < 0.05$.

or greater reductions in pH from 6.20 to 5.69 have been observed in other meat products fermented by the diversity starter culture lactobacilli (Gök et al., 2008; Kaban, 2013; Kaban and Kaya, 2006). After 36 months of ageing, the highest pH value (6.16) was noted in the control sample with sea salt and the lowest for the sample with nitrite (N) and the sample with acid whey (W). Amino acids released during proteolysis can be decarboxylated, deaminated, or even further metabolized (Table 3). Therefore, the ammonia and amines generated cause an increase in pH, which was observed during the last phase of ageing (Toldrá et al., 2001). pH is a key factor influencing microbiological safety and amino acid decarboxylase activity, and is also an important factor in the control of biogenic amine formation in fermented meat products. Teodorovic, Buncic, and Smiljanic (1994) showed that amino acid decarboxylases exhibit an optimum activity at pH between 4.0 and 5.5. Bover-Cid et al. (2006) pointed out that a rapid and sharp reduction in pH in sausage reduces the growth of the amine-positive microorganisms, particularly *Enterobacteriaceae* and protects the meat product from biogenic amine formation.

The ageing period had a significant effect ($p < 0.05$) on the water activity of fermented beef. The a_w values of fermented beef aged for 2 months were significantly lower ($p < 0.05$) than those for fermented beef aged for 21 days. Water activity decreased gradually during ageing with a mean value of 0.936 for samples aged for 2 months and 0.906 for samples aged for 36 months. The decrease observed in water activity confirms the findings in the literature (Gök et al., 2008; Kaban, 2013; Kaban and Kaya, 2006) that a systematic decrease in water activity in fermented meats results from a progressive protein proteolysis leading to a production of low-molecule compounds. The increase in FAA concentration (Table 3) during ageing confirmed this finding. The a_w value dropped to 0.90 in N and W samples at the end of ageing, which is a good hurdle effect for fermented beef. The absence of *S. aureus* and *L. monocytogenes* in the sample with nitrite (N) and the sample marinated with acid whey (W) confirmed this finding (Table 4). Marinating beef in acid whey affected ($p < 0.05$) ORP values during ageing time. A significantly higher ORP was observed in salted beef compared to the cured beef and marinated beef throughout the 36-month ageing period (Table 1). ORP values mainly increased significantly ($p < 0.05$) throughout the ageing period.

In the present studies it was found that TBARS values were affected significantly ($p < 0.05$) by the ageing time and the use of additives (Table 1). Significantly ($p < 0.05$) higher TBARS values were found in the salted beef sample (S) compared with the cured (N) and marinated ones (W) during the whole ageing period. The TBARS were found in the following order: S > N > W and their mean values were 4.32–1.20, 3.41–0.61 and 3.49–1.19 mg/kg respectively. These results indicated that marinating beef samples in acid whey were more effective against TBARS formation than nitrite. The rate of lipid oxidation was higher during the 21-day ageing than during the other periods. After 21 days' ageing the TBARS values decreased gradually, probably due to further decomposition of secondary lipid oxidation products to volatile compounds after 2 and 36 months of ageing.

The *N*-nitrosamines (N-nitrosodibutylamin, N-nitrosodiethylamin, N-nitrosodimethylamin, N-nitrosodipropylamin, N-nitrosomorpholin, N-nitrosopiperidin) yields in this study are very low and balancing at the limits of detectability of the method applied (0.5 µg/kg) after 36 month of ageing. The higher levels of biogenic amines (Table 4) which were investigated in salted samples S and W do not cause a nitrosamine increase. Similar results were also reached by Drabik-Markiewicz et al. (2011).

The evolution in the concentration of 16 free amino acids is depicted in Table 2 for the three ageing beef products. After 21 days of ageing the chief FAAs were glutamic acid (55.70–82.56 mg/100 g DM), alanine (52.93–69.52 mg/100 g DM), lysine (61.23–75.45 mg/100 g DM) and leucine (41.08–50.56 mg/100 g DM). After 21 days of ripening period, the FAA content in salted beef fermented spontaneously and was higher by about 38% in comparison with curing beef and beef marinated in acid whey. These four free amino acids represented 50% of the total amino acids by day 21. In the present study, a decrease in 5 FAAs (serine, glutamic acid, tyrosine, arginine) and an increase in other FAAs content was observed after 36 months of ageing.

Decreases in serine, glutamic acid. tyrosine and arginine were also reported by other researchers, especially during the extended ripening of dry-cured ham

Table 2. Free amino acids content in fermented beef examined after ripening (21 d) and after 36 months of ageing period

Amino acid	Storage month	Sample, mg/100 g DM		
		S	N	W
Threonine	0	30.0 ±4.2^{aA}	21.7 ±3.9^{bA}	20.5 ±4.1^{bA}
	36	41.1 ±7.0^{aA}	30.4 ±6.7^{aA}	31.2 ±7.0^{aA}
Serine	0	32.0 ±3.9^{aA}	26.1 ±3.8^{aA}	23.3 ±3.5^{bA}
	36	28.4 ±4.1^{aA}	46.2 ±6.6^{bB}	15.8 ±2.1^{cA}
Glutamic acid	0	82.6 ±8.2^{aA}	56.5 ±7.0^{bA}	55.7 ±5.9^{bA}
	36	43.8 ±6.3^{aB}	45.0 ±5.8^{aA}	45.8 ±5.7^{aA}
Proline	0	44.2 ±7.6^{aA}	14.6 ±2.3^{bA}	21.3 ±2.7^{cA}
	36	49.0 ±6.6^{aA}	49.4 ±6.2^{aB}	43.4 ±5.8^{aB}
Glycine	0	25.7 ±3.0^{aA}	18.6 ±2.8^{aA}	17.4 ±2.9^{aA}
	36	75.8 ±12.6^{aB}	48.6 ±6.4^{bA}	40.7 ±5.9^{bB}
Alanine	0	69.5 ±7.6^{aA}	59.2 ±6.1^{aA}	52.9 ±5.7^{aA}
	36	216.1 ±25.5^{aB}	138.2 ±21.9^{bB}	123.6 ±20.7^{bB}
Valine	0	41.1 ±4.4^{aA}	32.0 ±4.4^{aA}	33.2 ±4.6^{aA}
	36	119.7 ±14.3^{aB}	82.9 ±13.4^{aB}	69.1 ±5.3^{bB}
Isoleucine	0	29.2 ±2.3^{aA}	22.5 ±2.6^{aA}	22.9 ±2.4^{aA}
	36	79.8 ±11.8^{aB}	50.6 ±7.4^{bB}	47.8 ±4.5^{bB}
Leucine	0	50.6 ±5.1^{aA}	41.1 ±3.8^{aA}	42.7 ±4.1^{aA}
	36	128.8 ±13.4^{aB}	75.8 ±8.4^{bB}	88.1 ±8.3^{bB}
Tyrosine	0	23.3 ±3.9^{aA}	17.0 ±2.1^{aA}	10.7 ±1.6^{bA}
	36	7.1 ±1.4^{aB}	15.0 ±1.7^{bA}	5.5 ±1.2^{aB}
Phenylalanine	0	27.3 ±2.9^{aA}	20.9 ±3.0^{aA}	23.7 ±3.1^{aA}
	36	67.1 ±7.2^{aB}	50.2 ±6.4^{aB}	51.0 ±6.3^{aB}
Lysine	0	75.4 ±8.7^{aA}	60.4 ±7.9^{aA}	61.2 ±6.9^{aA}
	36	220.0 ±21.2^{aB}	163.5 ±19.3^{bB}	114.1 ±19,8^{bB}
Histidine	0	19.7 ±3.0^{aA}	13.8 ±2.9^{aA}	13.0 ±2.7^{aA}
	36	45.8 ±4.8^{aB}	36.7 ±4.7^{bB}	26.1 ±3.8^{cB}
Arginine	0	20.9 ±2.9^{a}	24.1 ±3.6^{a}	12.2 ±2.1^{b}
	36	n.d.	n.d.	n.d.
Citruline	0	13.4 ±1.6^{aA}	n.d.	7.1 ±1.4^{bA}
	36	15.8 ±1.7^{aA}	14.6 ±2.0^{a}	17.0 ±1.5^{aB}
Ornitine	0	n.d.	n.d.	6.7 ±0.7^{A}
	36	83.34 ±9.3^{a}	49.4 ±5.9^{b}	36.3 ±4.8^{cB}

Mean value ±standard error.
Samples: S – control with sea salt (3%), N – control with curing mixture (3%), W – experimental with acid whey and sea salt (3%).
n.d. – not detected.
Means followed by the same letters within columns (capital letters) and row (lower case letters) are not significantly different at $p < 0.05$.

(Alfaia et al., 2004). A gradual release of amino acids throughout ageing is typical in dry fermented meat products (Rabie et al., 2014; Stadnik and Dolatowski, 2015). Such increasing trends were typically observed for all study samples, and are consistent with reports by Stadnik and Dolatowski (2015). Among the amino acids, lysine, tyrosine and histidine are the main precursors for biogenic amines (Stadnik and Dolatowski, 2015). In the present study, these amino acids exhibited increases for lysine and histidine and decreases for tyrosine for all samples respectively after 36 months of ageing. Rabie et al. (2014) also reported considerable increases throughout storage of these three FAAs in the case of fermented horse meat and only one of them (lysine) in the case of beef products. The TFAA content was higher for the salted beef sample (S) and the cured one (N) compared with salted beef marinated with acid whey (W) after 21 days and 36 months of ageing. The average total amino acid content increased from 584.99, 428.58 and 421.07 mg/100 g DM after 21 days of ageing to 1228.84, 896.65 and 755.63 mg/100 g DM at the end of the longest ageing period for the salted, cured and marinated beef samples, respectively.

The concentration of 7 biogenic amines is depicted in Table 3. The highest concentration of total biogenic amine (i.e. 1159.0 mg/kg) was found in fermented beef marinated with acid whey, whereas a total of only 209.8 mg/kg was observed in control beef with nitrite. The beef marinated with acid whey has a total biogenic amine concentration higher than 1000 mg/kg, which is considered as a heuristic rule in terms of the danger to human health (Silla-Santos, 1996). The higher concentration of BA in W samples correlated well with the lower level of FAAs observed. The content of Tyr, His, Lys, Arg in beef marinated with acid whey was lower compared with cured beef. Several authors working with Spanish fermented meat products found that tyramine and cadaverine could reach 600 mg/kg, putrescine 450 mg/kg and tryptamine up to 50 mg/kg. Tyramine and putrescine were the most abundant BAs which were found in fermented meat products. Rabie et al. (2010) reported that BA contents in Egyptian fermented meats ranged from 277 to even 5815 mg/kg within 30 days of storage. Papavergou et al. (2012) noted the concentration of this BA ranging from 0 to 505 mg/kg. Similar putrescine concentrations (159.4 mg/kg) were found in spontaneously fermented sausages produced in Italy compared with beef marinated in acid whey (139.50 mg/kg). The histamine was not detected in any of the products. The permitted limits for histamine are 40–100 mg/kg and

Table 3. Concentration of biogenic amine in fermented beef examined after 36 months of ageing period, mg/kg

Biogenic amine	Sample, mg/kg		
	S	N	W
Histamine	n.d.	n.d.	n.d.
Tyramine	369.5 ±74.25^{a}	62.60 ±3.39^{b}	437.50 ±57.28^{a}
Putrescine	57.1 ±1.13^{a}	85.20 ±8.06^{b}	139.50 ±2.21^{c}
Cadaverine	244.0 ±16.97^{a}	62.00 ±6.08^{b}	582.0 ±14.14^{c}
Spermidine	n.d.	n.d.	n.d.
Agmantine	n.d.	n.d.	n.d.
Spermine	n.d.	n.d.	n.d.
Total biogenic amines	670.6	209.8	1 159.0

Mean value ±standard error.
Samples: C – control with sea salt (3%), N – control with curing mixture (3%), W – experimental with acid whey and sea salt (3%).
n.d. – not detected.
Means followed by the same letters within row (lower case letters) are not significantly different at $p < 0.05$.

Table 4. Amount of lactic acid bacteria and selected pathogenic bacteria in fermented beef examined during ageing period (36 months)

Sample	Time month	Log cfu/g			
		LAB	*TVC*	*S. aureus*	*L. monocytogenes*
S	0	5.64 ±0.01^{aA}	6.44 ±0.01^{aA}	<10	<10
	36	6.30 ±0.03^{aB}	6.29 ±0.02^{aB}	1.18 ±0.02^{a}	1.16 ±0.02^{a}
N	0	5.75 ±0.04^{bA}	6.27 ±0.07^{bA}	<10	<10
	36	6.27 ±0.02^{aB}	6.27 ±0.03^{aA}	<10	<10
W	0	5.67 ±0.04^{aA}	6.46 ±0.02^{aA}	<10	<10
	36	5.89 ±0.02^{bB}	6.02 ±0.01^{bB}	<10	<10

Mean value ±standard error.
Samples: S – control with sea salt (3%), N – control with curing mixture (3%), W – experimental with acid whey and sea salt (3%).
Means followed by the same capital letters (storage time) and by the same lower case letters (treatments) are not significantly different at $p < 0.05$.

the levels above 100 mg/kg may cause some manifestation of poisoning. Rabie et al. (2014) found that day 28 histamine in beef sausage was on the level of 31.24 mg/kg. The other author found a histamine level from 6.72 to 768 mg/kg in different kinds of fermented meat products (Ansorena et al., 2002; Rabie et al., 2010; Şenöz et al., 2000). A higher ($p < 0.05$) concentration of tyramine was noted for salted (S, W) fermented beef 369.5 mg/kg and 437.50 mg/kg respectively compared with cured (62.60 mg/kg). Some *Lactobacillus* and *Enterococcus* strains have been identified as tyramine producers because of their tyrosine decarboxylating activity (Papavergou et al., 2012). A lower level of tyramine was found by other scientists (Ansorena et al., 2002; Rabie et al., 2010; Rabie et al., 2014). Shalaby (1996) proposed a tyramine level from 100 to 800 mg/kg and a histamine level from 50 to 100 mg/kg as fermented products compatible with GMP. Furthermore, the EFSA states that daily maximum intake of tyramine should not exceed 600 mg/kg. The highest content of cadaverine (582.0 mg/kg) was found in beef marinated with acid whey, whereas the lowest (62.00 mg/kg) was detected in cured beef samples. It can be concluded that some LABs present in acid whey are amine-positive. This could explain the highest BA content in beef marinated in acid whey. The cadaverine concentration in other fermented meat products ranged from 0 to 690 mg/kg especially in fuet. chorizo and saucissons (Papavergou et al., 2012; Suzzi and Gardini, 2003). Finally, spermine, spermidine and agmantine were not detected in any of the study samples (Table 4).

Pathogenic bacteria and other microorganisms are known to be the most important food safety hazards associated with ageing meat products. Pathogens that are likely to be found which are associated with fermented meat are *Stahylococcus* spp., *Cl. botulinum*, *Salmonella* spp., *Vibrio* spp. and *E. coli*.

The production of safe fermented meat products is important to consumers and depends on both science and experience. In relation to science, dangerous microorganisms which can be involved in the fermentation of meat must be identified. As shown in Table 4, the level of LAB after 21 days of ripening was similar in all study samples (5.64–5.75 log cfu/g). After 36 months of ageing, the number of LAB stood at a level from 5.89 to 6.30 log cfu/g. At the end of the ageing period, the LAB count in samples marinated with acid whey was lower by approximately one logarithmic level than controls with sea salt (S). *S. aureus* and *L. monocytogenes* were not detected in two samples N and W after beef ripening as well as after 36 months of ageing. Only in salted beef did we discover the presence of *S. aureus* on a level of 1.18 log cfu/g and *L. monocytogenes* on a level of 1.16 log cfu/g. During ageing, the total viable count (TVC), which indicates the general product quality, decreased in all beef

samples at the end of the ageing period. The level of TVC after ripening was 6.27 to 6.46 log cfu/g in all samples.

CONCLUSIONS

In this study, the microbiological presence in acid whey did not inhibit the production of biogenic amines in the samples analyzed. The high concentration of free amino acids, the potential precursors of BA, could be attributed to intense peptidase activity. Histamine was not detected in either of the products. The highest concentration of total biogenic amine (i.e. 1159.0 mg/kg) was found in fermented beef marinated with acid whey, whereas a total of only 209.8 mg/kg was observed in control beef with nitrate and nitrite. However, a lactic acid bacteria strain from acid whey reduced the pH value during the first steps of ageing and ensured the microbiological safety of the product not only in the first stage of fermentation but also at the end of ageing (36 month). The *N*-nitrosamines were not detected in any of the aged beef samples.

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