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Research Article

Spontaneously Fermented Beef Produced According to Traditional Recipe used in the Middle East without Nitrite

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Abstract

The aim of the study was to evaluate the quality (pH, water activity, color parameters, biogenic amines, microbiological analysis) of spontaneously fermented beef produced without the addition of nitrite. The effect of six month storage was also evaluated. After the end of production, the product was characterized by an average pH of 5.43 and a low water activity (0.813). The research performed showed no significant changes in pH, water activity, color and microbiological characteristics after six months of fermented beef storage. Total concentration of biogenic amines in fermented beef produced according to traditional recipe used in Middle East was in the range of 52.05 – 54.06 mg kg-1 product. Examined products contained two biogenic amines, namely spermidine and spermine. Spermine was found to be the dominant amine in examined fermented beef.

INTRODUCTION

Fermented beef covered with fenugreek paste, called pastrami, is a traditional product produced in Turkey and in other Middle East countries [1]. Pastrami is traditionally produced from whole muscle obtained from certain parts of beef carcasses, mainly *M. longissimus dorsi*, however, various pastrami types are produced from different muscles and they are named according to the location of the muscle (e.g. bohça, kürek, sırt) [2]. As stated by Karabıyıklı et al. [3], pastrami is defined as "a cured, dried and non-thermally heated meat product which is produced from beef by curing and washing and then pressing and curing and then finally covering with fenugreek paste and drying" in Turkish Food Legislation. Therefore, one of the most important factors influencing the quality and safety of the fermented beef is the addition of nitrogen compounds during curing [4]. Nitrogen compounds addition in meat technology is a process applied to improve the characteristics such as appearance, color, texture and flavor of the product. The formation of nitrosyl myoglobin, with the participation of nitric oxide resulting from the reduction of nitrites, generates the cured meat product color. Nitrates/nitrites also show antioxidant and antimicrobial properties. Antimicrobial effect is related to inhibiting metabolic enzymes of bacteria, limiting oxygen uptake, and breaking the proton gradient. Nitrites and nitrates act against lipid oxidation through the oxygen deletion or binding transitional metals [5].

Many scientific research concerns the elimination of nitrogen compounds from the recipes of various meat products in order to increase their nutritional value [6-8]. This action is also considered a strategy to improve the nutritional value of meat products [9]. However, studies about the effects of nitrite reduction or elimination on the quality parameters of pastrami are limited [10, 11].

Another factor that determines the quality of fermented beef produced according to traditional recipe used in the Middle East is coating meat during processing with fenugreek paste which is called as "çemen". This process contributes to the characteristic taste and aroma to pastrami as well as physicochemical and microbiological profile of the end product. As reported by Isikli and Karababa [12], the fenugreek paste is effective against microbial contamination and it serves as a barrier.

It has been hypothesized that it is possible to obtain good quality fermented beef produced on the basis of traditional recipe used in the Middle East without addition of nitrogen compounds. The aim of the study was to evaluate the effect of 6-month storage on the quality characteristics (pH, water activity, color parameters, biogenic amines, lactic acid bacteria and *Enterobacteriaceae* count) of fermented beef produced without the addition of nitrite.

MATERIALS AND METHODS

Fermented beef production

Fermented beef produced in Department of Meat Technology and Food Quality University of Life Sciences in Lublin according to traditional recipe used in Middle East was the examined material. The pieces (about 1 kg each) of beef meat (*M. biceps femoris* muscle) have been taken. Pieces have been cleaned well from

the remains of unwanted fat. After that clean sea salt has been added to the meat pieces from all sides by 150-200 g kg^{-1}. Pieces of the meat have been put on the buckle and put the weight above them to accelerate the removal of succulents (blood). Salting was carried out for 48 hours in the refrigerator. After 48 hours, pieces of meat have been taken out and washed with cold water quickly to get rid of excess salt and then drying in ripening chamber at 18 °C for 24 hours. At the next day the "çemen" has been done which is characteristic of this product consisting of powder fenugreek 150 g, garlic 100 g, fresh red pepper paprika 80 g, hot red pepper 10 g, black pepper 15 g, cumin 15 g, coriander 15 g and 40 ml of water. All spices have been placed in a blender and mixed until get cohesive dough. Then pieces of meat have been covered by a paste of spices from all sides properly without leaving a gap to maintain product. After that pieces of meat have been suspended in the drying device and fermented at 22°C for a period of 10 days. Fermentation was carried out spontaneously without any starter cultures. The production scheme of fermented beef is shown in Figure 1.

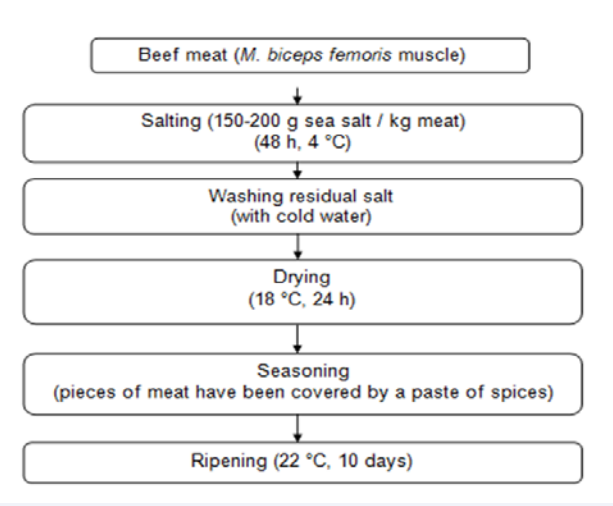

Figure 1 Production of fermented beef.

Sample collection

Meat products were evaluated at the end of production (pH, water activity and color parameters) and after 6 months of storage under vacuum at temperatures 4°C (pH, water activity, color parameters, biogenic amines, microbiological analysis). The treatments were replicated twice by producing two different batches. Each sample was analyzed in triplicate.

The Physicochemical Parameters (Water Content, pH, and Water Activity)

The water content (WC) was determined according to PN ISO 1442:2000 [13]. A digital pH meter CPC-501 (Elmetron, Zabrze, Poland) equipped with a temperature sensor and pH electrode (ERH-111, Hydromet, Gliwice, Poland) was used to measure pH of the samples. The water activity (a_w) was measured using a water activity analyzer calibrated with Novasina SAL-T humidity standards (Novasina AG, Lachen, Switzerland). Measurements were made at a temperature of 20 °C.

Instrumental Color Measurement

Color parameters were determined on the cross-section (about 20 mm thick) just after the sample was cut [14]. Color parameters (L*, a*, b*) were measured using an X-Rite 8200 colorimeter (X-Rite, Inc., Michigan, USA) calibrated using the black glasses and white tiles provided. Samples were analyzed directly over the 12 mm aperture, with D65 illumination configurations and 10 viewing angles.

Microbiological analysis

Enterobacteriaceae were quantified by means of the colony-count technique [15]. The number of Lactic Acid Bacteria (LAB) was determined according to PN-ISO 15214:2002 [16]. The counts were expressed as colony-forming units (log CFU g^{-1}). Microbiological analyses were carried out at the Eurocontrol Laboratory (Dęblin, Poland).

Biogenic Amines (BAs) Determination

Biogenic amines (BAs) were determined in fermented beef samples after extraction using 10% trichloroacetic acid. The analysis of BAs was performed using an AAA 500 amino acid analyzer (Ingos, Praha, Czech Republic), equipped with an Ostion LG AAA8 ion-exchange column (3.6 × 100 µm, 8 µm). Standards of BAs with a purity of 99% were obtained from Sigma-Aldrich (St. Louis, MO, USA). The volume of the injected sample was 100 µL. The reactor temperature was set to 120 °C. The content of the BAs was determined with a reference to the amine standards, which were supplied by Ingos, Czech Republic. The BA concentrations were reported as mg kg^{-1} of product.

Statistical analysis

The experiment was replicated twice with separate preparation of meat product batches. Three replicates per batch were analyzed. The results were expressed as mean ± standard deviation. One-way analysis of variance at $p< 0.05$ was used to analyze the effect of storage time. The significance of differences between the sample at different ripening times was determined using Tukey's test using Statgraphics v. 5 (Manugistics Inc., Rockville, MD, USA).

RESULTS AND DISCUSSION

The shelf-life and microbiological safety of fermented beef produced according to traditional recipe used in Middle East are mainly due to low water activity of the end product and microbial changes during ripening. Combined effects of drying and covering with fenugreek paste on the final product lead to protection and preservation. The experimental samples of fermented beef produced without nitrogen compounds were characterized by a low water content, averaging 52.47% after the end of production (Table 1). The water content did not change statistically significantly after 6 months of storage due to the application of *vacuum* conditions. Aksu et al. [11] obtained a lower water content of the final product produced with different (50-150 ppm) addition of nitrite.

The pH value of fermented beef at the end of production was 5.43, which confirmed the effectiveness of the fermentation process of acidification of beef. The high LAB content confirmed

Table 1: The effect of storage on water content, pH and water activity values of fermented beef.

	WC (%)	pH	aw
At the end of production	52.47 ± 3.96[a]	5.43 ± 0.11[a]	0.813 ± 0.027[a]
After 6 months of storage	53.12 ± 1.05[a]	5.50 ± 0.02[a]	0.828 ± 0.015[a]

Means followed by the same letters do not differ significantly (p >0.05)

this finding (Table 3). After six months of storage, the pH of uncured fermented beef did not change significantly. Higher pH have been observed by Aksu et al. [11] in pastirma. They indicated that the average pH values of meat products were difference depending on the nitrite levels. Similar or higher pH was also noted in other meat products fermented by the diversity starter culture lactobacilli [2, 17]. Higher pH values were obtained by Wójciak and Solska [18] for fermented beef after the ripening process lasting 21 days (5.96 - 5.74).

The production process used in the current study made it possible to obtain low water activity of the fermented beef samples (average 0.813 and 0.828, at the end of production and at six months of storage respectively), which positively influences their microbiological stability. After the storage period, an increase in the water activity of the samples was observed, which was probably related to the proteolysis process. However, the changes were not statistically significant.

Color parameters, mainly redness (a*) is the most important quality attributed for nitrite-free meat products. The L* values of experimental uncured fermented beef ranged from 35.5 at the end of production to 37.1 after six months of storage (Table 2). Similar lightness was also noted in other fermented beef produced according to traditional recipe used in Middle East [19-20]. The a* parameter obtained in the current study for uncured fermented beef was lower compared to study performed by Aksu et al. [11] probably due to the type of raw material used. Aksu et al. [11] indicated that the a* color parameter of pastirma increased with the addition of nitrite. Similar to our results, Kęska et al. [21] showed a* values in the range of 7.22 - 9.40 for organic dry-fermented beef.

In fermented meat products acidification and proteolysis occurring during fermentation make the environment favorable for biogenic amine accumulation [22]. The accumulation of BA depends on the microorganisms with amino acid decarboxylases, amino acids precursors, and favorable conditions for growth and decarboxylation. Conditions may vary depending on different extrinsic and intrinsic factors during the manufacturing process, redox potential, such as temperature, NaCl, pH, the size of the sausage and hygienic conditions of manufacturing practices [23]. Determination of the biogenic amine content in foods is of interest because of its toxicological implications since some biogenic amines can pose health risks when ingested in high quantities [24]. The amounts of biogenic amines in examined fermented beef produced according to traditional recipe used in Middle East are reported in Table 3. Examined products contained two biogenic amines, namely spermidine and spermine. Histamine was not detected what can be attributed to the fact that bacteria with capacity to decarboxylate histidine are uncommon in meat product. The same phenomenon has been observed with dry-cured pork loins [24]. Spermine was found to be the dominant amine in examined fermented beef (average 46.79 mg kg^{-1} and 47.15 mg kg^{-1}, at the end of production and at six months of storage, respectively). These values are higher than those reported by Stadnik and Dolatowski [25]. The second biogenic amine was spermidine, which showed an average of 6.48 mg kg^{-1} at the end of production. Total concentration of biogenic amines was in the range of 52.23 – 53.27 mg kg^{-1} product and was similar to those obtained by Stadnik and Dolatowski [25] for control sample produced without probiotic strain. Much higher amounts of biogenic amines for beef was noted by Wójciak and Solska [18]. In their study concentration of total biogenic amine in fermented beef was from 209.8 for sample with nitrate and nitrite to 1159.0 mg kg^{-1} for nitrite-free samples.

Biogenic amines are indicators of the microbiological quality of food, thus their identification and quantification are important [26]. As reported by Barbieri et al. [27], LAB and *Enterobacteriaceae* are capable of forming biogenic amines, though the LAB are the main producers. Therefore, samples with a higher bacterial population are expected to contain more amines. In our study, the number of *Enterobacteriaceae* in uncured fermented beef was low both after the end of production and after six months of storage, similar to the results obtained by Aksu et al. [11]. They obtained <2 log CFU g^{-1} of *Enterobacteriaceae* for pastirma with the level of nitrite in the range of 0-150 ppm. Our results showed higher lactic acid bacteria counts in uncured fermented beef (average 5.99 log CFU g^{-1} at the end of production) compared to pastirma cured with different levels of sodium nitrite [11] which resulted in a lower pH as mentioned earlier.

Table 2: The effect of storage on color parameters (L*a*b*) of fermented beef.

	L*	a*	b*
At the end of production	37.1 ± 2.7[a]	5.3 ± 0.5[a]	3.4 ± 0.5[a]
After 6 months of storage	35.5 ± 4.1[a]	6.3 ± 1.6[a]	2.7 ± 1.1[a]

Means followed by the same letters do not differ significantly (p > 0.05)

Table 3: The effect of storage on the concentration of biogenic amines, lactic acid bacteria and *Enterobacteriaceae* counts in fermented beef.

	At the end of production	After 6 months of storage
Results of microbial analysis (log CFU g^{-1})		
Lactic acid bacteria counts	5.99 ± 0.15[a]	5.67 ± 0.29[a]
Enterobacteriaceae counts	1.00 ± 0.16[a]	1.34 ± 0.28[a]
Biogenic amines concentration (mg kg^{-1})		
Cadaverine	nd	nd
Histamine	nd	nd
Putrescine	nd	nd
Spermidine	6.48 ± 0.04[b]	5.08 ± 0.09[a]
Spermine	46.79 ± 1.09[a]	47.15 ± 1.21[a]
Total	53.27 ± 1.06	52.23 ± 1.15

Means followed by the same letters do not differ significantly (p > 0.05)

The six-month storage did not significantly change the number of these bacteria, which resulted in a stable pH of the products during storage.

CONCLUSION

The elimination of nitrates from the production of fermented beef produced according to traditional recipe used in Middle East gives a chance to produce a meat product with increased nutritional value. The research has shown that the production process used, including drying and covering with fenugreek paste, may be sufficient to obtain a safe product. It was indicated that the final product is stable over the assessed six month storage period.

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