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Analysis of vacuum packed beef regarding psychrotrophic bacteria growth and biogenic amines content

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Abstract

It has been recognized that biogenic amines (BA) content in meat can be considered a freshness marker. Considerable amounts of some BAs can appear during food storage under certain conditions, according to the handling of the raw material, technology applied, storage temperature and time, packaging condition, mainly if amino acid - decarboxylase positive microorganisms are present. The aim of this study was to evaluate the psychrotrophic bacteria growth and metabolic production of BAs during chill storage of beef. The vacuum packed beef cuts (*Longissimus dorsi* muscle) were analyzed during storage at 7 °C at 0, 15, 30, 45, and 60 d, to determine the psychrotrophic bacteria growth and the BAs amount. The BAs considered were: putrescine, cadaverine, histamine, spermidine, and spermine. The BAs quantitative determination was carried out by reversed phase high-performance liquid chromatography (HPLC) with UV detection. Statistic procedures were performed using SAS statistical software. The growth parameters of psychrotrophic bacteria including lag phase, maximum specific growth rate, maximum bacterial cell density, initial population, mean square error, and coefficient of determination were determined according to Baranyi and Roberts model. The values of histamine and spermidine increased significantly ($P < 0.0001$) during storage time, while the levels of spermine decreased ($P < 0.0001$). Psychrotrophic bacteria counts increased significantly ($P < 0.0001$) reaching 7.6 log cfu/g over time. The counts of this group positively correlated to histamine and spermidine ($r = 0.68$ and 0.61, respectively), while spermine showed a negative correlation ($r = -0.70$). Conversely, no significant correlation was found between psychrotrophics counts and putrescine or psychrotrophics counts and cadaverine.

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Keywords: meat, psychrotrophic bacteria, biogenic amines, predictive modeling, storage

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1. Introduction

BAs, such as histamine, tyramine, cadaverine, putrescine, spermine, and spermidine, are low molecular weight organic bases that present biological activity. They are found in a variety of foods, including meat and meat products, in which BAs can be generated by enzymatic pathways, mainly through the descarboxylation of free amino acids produced by the action of microorganisms presented at the food or by the meat tissues themselves. The presence of these compounds is of concern in relation to food safety and spoilage.

BA production requires the presence of free amino acids (type of meat, raw material quality), the descarboxylase enzyme (types and proportion of microbial population with descarboxylase activity), manufacturing processes and practices (fermentation, heat treatment) and appropriate environmental conditions (time/temperature, packaging, temperature abuses)[1,2]. When these factors are combined, they will determine the final amount of BAs present in the food. BA formation is temperature dependent as it is increased at high temperatures of storage since this parameter can favor the growth of bacteria[3]. Heating can destroy BAs-producing bacteria in food; however, BAs are heat-stable, so applying heat after the production of these compounds in the food will not be effective in eliminating it[1].

The aim of this work was to evaluate the relation between the growth of psychrotrophic bacteria and the formation of BAs on vacuum-packaged chilled beef cuts stored at 7°C.

2. Materials and methods

2.1. Preparation of beef samples, storage conditions and sampling

Samples of beef cuts (M. *Longissimus dorsi*) were obtained from carcasses of animals *Bos indicus* stored at 4 °C for 2 d after slaughtering. The muscles were cut in similar size (100 g, 1 cm thickness), vacuum-packaged in high density polyethylene barrier film bags (Cryovac®, B-2620) and stored at 7 °C during 60 d. The analyses were performed at days 0, 15, 30, 45, and 60 d. The reported values are the mean of nine determinations of three portions obtained from three animals.

2.2. Bacterial counts

Total psychrotrophic bacteria growth was determined[4]. Samples (25 g) of beef were aseptically weighed and homogenized in 225 mL of 0.01% (w/v) sterile peptone water solution in a stomacher. Decimal dilutions were prepared, and aliquots of 0.1 mL of the appropriate dilutions were spread plated in duplicate into the Plate Count Agar, and incubated at 7 °C for 10 d under aerobic conditions.

2.3. Quantification of the BAs

The beef samples were quantitatively analysed for putrescine, cadaverine, histamine, spermidine, and spermine. According to a modification of the methodology of Malle et al.[5], 5 g of homogenized beef samples were extracted with 10 mL of 0.2 M perchloric acid, following homogenization and centrifugation. 400 µL of the supernatant were collected and 800 µL of saturated sodium bicarbonate solution were added. Derivatization step was performed with 0.75% dansylchloride in a water-bath at 60 °C for 5 min. This was followed by the addition of 10% L-proline solution, then the solution was left to stand in dark at room temperature for 30 min. After the addition of 2 mL of toluene for phase separation, the organic phase was recovered and evaporated. Acetonitrile was added and the solution was filtered in PTFE membrane, followed by analysis in HPLC (Shimadzu) with C18 reverse phase column (5 µm, 100 Å, 25 cm x 4.6 mm), detector UV at 254 nm, using injection of 20 uL. The chromatograms obtained from the samples were compared with chromatograms of standard solutions, and the analyte peaks were confirmed through time of retention. The total peak area of each analyte was interpolated on a standard curve relating the total area with the concentration of analyte.

2.4. Statistical methods

Statistic procedures were performed using SAS V9.1[6] statistical software. The bacterial growth parameters, including lag phase, maximum specific growth rate, initial population, mean square error, maximum bacterial cell density and coefficient of determination (R^2) in the primary model, were determined according to Baranyi and Roberts[7] model.

3. Results and discussion

Psychrotrophic bacteria increased significantly ($P < 0.0001$) reaching counts higher than 7.0 log cfu/g over time (Table 1). As shown in Figure 1 the Baranyi and Roberts primary model fitted with the growth of psychrotrophic bacteria on vacuum-packaged chilled beef cuts stored at 7 °C during 60 d. It produced a typical S-type growth curve that described how the changes of the bacterial concentration happened under this particular condition. The kinetic parameters were: R^2 0.943, mean square error 0.551, initial population 2.0995 (±0.55) $\log_{10}$ cfu/g, maximum bacterial cell density 7.667 (±0.657) $\log_{10}$ cfu/g, lag phase 102.694 (± 236.653) h, and maximum specific growth rate 0.00502 (±0.0017) h^{-1}.

The concentration of histamine and spermidine increased significantly ($P < 0.0001$) throughout the entire storage time, while the levels of spermine decreased ($P < 0.0001$). The amount of spermine was high since the first day of storage, it is relevant to note that this compound and spermidine are naturally present in meat, and their production are attributed to physiological reactions of the food and not to the bacterial activity[8]. Some authors[9,10,11] also related the decrease in the levels of spermine in beef samples, during the storage and this fact could be attributed to the utilization of this compound as nitrogen source to the growth of microorganisms. High amounts of spermine, between 20 and 60 mg/kg, are usual in meat[3]. In relation to cadaverine and putrescine, no statistical difference concerning their concentration was observed during the storage. Nevertheless, the results average indicated a high variation among the gross values (data not shown), with a tendency for both BAs to increase their amounts along the storage time (considering the nine samples analyzed). The maximum mean value of BAs obtained was from cadaverine reaching more than 30 mg/kg at 45 d of storage.

Table 1. Determination of psychrotrophic bacteria (log cfu/g) and quantification of BAs (mg/kg) on vacuum-packaged chilled beef cuts stored at 7 °C during 60 d.

Storage time (d)	Psychrotrophic bacteria (log cfu/g)	Biogenic amine (mg/kg)				
		Putrescine	Cadaverine	Histamine	Spermidine	Spermine
1	2.12 (0.00)^{e}	0.39 (0.01)^{a}	0.00 (0.02)^{a}	5.06 (0.25)^{c}	3.19 (0.10)^{b}	27.76 (0.28)^{a}
15	3.24 (0.02)^{d}	3.60 (0.86)^{a}	3.95 (1.08)^{a}	10.62 (0.26)^{bc}	5.36 (0.15)^{a}	27.63 (0.40)^{a}
30	5.60 (0.00)^{c}	9.77 (1.49)^{a}	6.18 (1.15)^{a}	11.76 (0.28)^{b}	5.86 (0.09)^{a}	22.97 (0.15)^{b}
45	6.53 (0.00)^{b}	25.99 (8.02)^{a}	32.43 (10.22)^{a}	13.51 (0.40)^{b}	5.40 (0.12)^{a}	24.42 (0.27)^{ab}
60	7.65 (0.02)^{a}	23.22 (7.08)^{a}	28.92 (8.45)^{a}	19.35 (0.84)^{a}	6.55 (0.15)^{a}	16.61 (0.48)^{c}
P-value	<0.0001	0.64	0.58	<0.0001	<0.0001	<0.0001

Mean (standard error); n= 9

Different letters in the same columns indicate significant differences ($P < 0.05$)

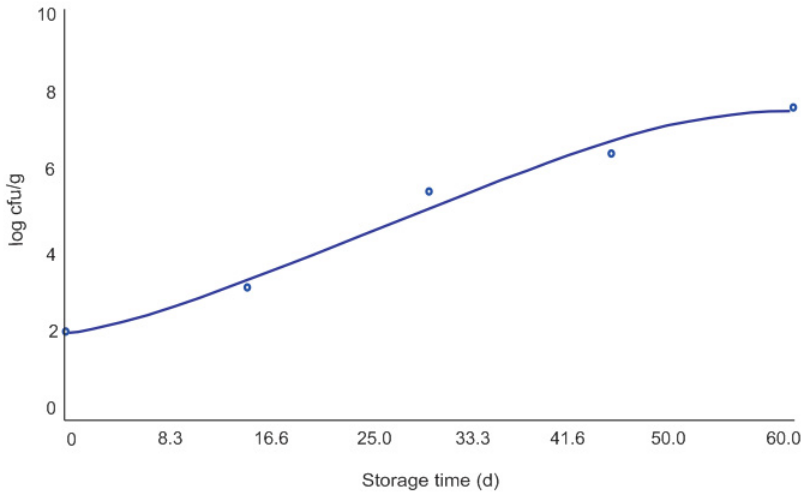

Fig. 1. Growth data of psychrotrophic bacteria and the fitted Baranyi and Roberts curve on vacuum-packaged chilled beef cuts stored at 7 °C during 60 d.

Psychrotrophic bacteria counts positively correlated to histamine or spermidine, while there was a negative correlation with spermine (Table 2). Conversely, no significant correlation was found between psychrotrophics counts and putrescine, or the bacteria counts and cadaverine. A high correlation was reported between the amount of cadaverine and putrescine, and it is important to note that some samples (data not shown) presented similar amounts of both BA.

Table 2. Pearson correlation coefficients between psychrotrophic bacteria and BAs, or the correlation between the BAs themselves on vacuum-packaged chilled beef cuts stored at 7 °C during 60 d.

	Psychrotrophic bacteria	Putrescine	Cadaverine	Histamine	Spermidine	Spermine
Psychrotrophic bacteria	1.00	0.21^{ns}	0.21^{ns}	0.68^{***}	0.61^{***}	-0.70^{***}
Putrescine		-	0.99^{***}	0.28^{*}	0.06^{ns}	-0.19^{ns}
Cadaverine		-	-	0.28^{*}	0.04^{ns}	-0.21^{ns}
Histamine		-	-	-	0.46^{***}	-0.46^{***}
Spermidine		-	-	-	-	-0.25^{*}
Spermine						1.00

ns: not significant; * p<0.1; ** p<0.05; *** p<0.001

4. Conclusions

This work showed that histamine is a possible indicator of the microbiological load in chilled beef and could be useful to control storage. Although spermidine and spermine presented a high correlation with the psychrotrophic bacteria counts, their natural occurrence in meat makes them inappropriate indicators of beef freshness. Considering the high variability of the results of quantification of putrescine and cadaverine, it is important to analyze a great number of samples in the same lot of beef in order to achieve an accurate result.

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