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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.