# Evaluation of the use of probiotic acid lactic bacteria in the development of chicken hamburger

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## Abstract

The objective of this study was to investigate the effect of fermentation with *Lactobacillus acidophilus* CRL 1014 on the physicochemical, microbiological and sensory characteristics of a hamburger product like processed with chicken meat and okara flour, with reduction of curing salts. A mixture of ingredients containing 90% chicken meat and 10% okara flour was subjected to the following treatments: F1: fermented with *Lactobacillus acidophilus*; F2:75 mg nitrite/kg and fermented with *Lactobacillus acidophilus*; F3: 150 mg nitrite/kg and unfermented. The quality of the "hamburgers" was assessed by physical and chemical analysis (pH, cooking yield and shrinkage), chemical composition, microbiological tests (*Salmonella* spp., count of sulphite-reducing clostridia, staphylococos coagulase-positive, total coliforms and *Escherichia coli*) and sensory analysis (sensory acceptance and purchase intent). During the first six days of fermentation, there was a decrease in pH from approximately 6.33 to 5.10. All the samples showed the same chemical composition (p < 0.05). The fermentation process was observed to inhibit the multiplication of microorganisms of several groups: coagulase-positive staphylococci, sulphite-reducing clostridia, *Salmonella* spp. and *E. coli*. The different "hamburgers" formulations showed high scores for all the sensory attributes evaluated, without differing from each other (p < 0.05). The results showed that the use of *L. acidophilus* CRL 1014 enabled the production of a safe product, with good physicochemical and sensory characteristics, in the absence of curing salts.

## Introduction

In light of the current requirements of the consumer market, it has become important to diversify the production of meat products. Thus, chicken has been industrialized as products previously produced with beef and pork, such as sausage, bologna, smoked sausage, ham and hamburgers. The use of okara, a by-product of soy aqueous extract, rich in fiber and protein, could also assist in obtaining differentiated meat products (Turhan *et al*., 2007). In Brazil, the supply of fermented meat products is limited, since this segment is dominated by sausage products. Fermentation is considered an effective way to increase the shelf life of foods and beverages through the action of microorganisms and their metabolites (Ross *et al*., 2002).

Among the important variables in the processing of fermented meat products, there is the choice of starter culture, which should optimize production time, assist in preservation by producing compounds with antimicrobial activity and improve the sensory characteristics of the product (Smulders *et al*., 1986; Lücke, 2000; Casamuri *et al*., 2005;

De Vuyst *et al*., 2008). Starter cultures typically used in the fermentation of meat products include bacteria belonging to the genera *Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp., *Leuconostoc* spp., *Pediococcus* spp. and *Lactobacillus* spp. owing to their ability to reduce the pH and to confer desirable sensory properties on the food (Verluyten *et al*., 2003).

Traditionally, meat products are prepared with curing salts (nitrite and nitrate) in order to inhibit the growth of pathogens, particularly *Clostridium botulinum*, a bacterium highly resistant to heat treatment and able to produce a potent neurotoxin. However, the residual nitrite present in meat may react with amines and amino acids, giving rise to nitrosamines, which are associated with the development of cancer and other chronic degenerative diseases (De La Monte *et al*., 2009).

Thus, the use of curing salts has been reconsidered and drastic reductions have been suggested in the levels of nitrite and nitrate in meat products (Tompkin, 2005; Sebranek and Bacus, 2007). Food fermentation with probiotic bacteria has been widely studied in order to improve the safety and acceptance

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of the final product, providing a viable means of reducing curing salts in meat products (Tompkin, 2005; Sebranek and Bacus, 2007; De Vuyst *et al.*, 2008). Several authors have reported the use of various strains of *Lactobacillus* spp. as starter cultures in the manufacture of meat products, in an attempt to control or inhibit the growth of spoilage and pathogenic microorganisms (Pidcock *et al.*, 2002; Kingberg *et al.*, 2005).

Previous studies have shown that the strain of *Lactobacillus acidophillus* CRL 1014 has the capacity to develop in the presence of bile salts and in acid conditions, while reducing cholesterol added to the culture medium. The same strain has been successfully used to produce a frozen "yogurt" with soybean; however, the effectiveness of this organism as a starter culture in meat products has not been tested (Rossi *et al.*, 1994; Miguel *et al.*, 2009). With the above considerations in mind, the objective of this study was to investigate the effects of fermentation with *Lactobacillus acidophilus* CRL 1014 on the microbiological safety and the physicochemical and sensory characteristics of a hamburger-like product processed from chicken and okara flour, with a reduced level of curing salts.

## Material and Methods

### Material

To prepare the hamburger, muscle of chicken fillet, purchased in the local market (Araraquara-SP) was ground and mixed with dry okara flour, a by-product of soy aqueous extract, produced at the Unit of Production and Development of Soy Products (Unisoja, Araraquara, SP, Brazil). The formulations also contained dextrose (Synth, São Paulo), microfine cellulose (Rhoester, Vargem Grande Paulista), a fat substitute (Simplesse® Dry 100, CP Kelco, Limeira, SP, Brazil), sodium nitrite (Synth, São Paulo, Brazil), sodium nitrate (Synth, São Paulo, Brazil) and BHT (Synth, São Paulo, Brazil). The other ingredients (seasonings, garlic powder, anion) were all purchased in the local market. The starter culture was a probiotic strain *Lactobacillus acidophilus* CRL 1014, from the Reference Center for *Lactobacilli* (CERELA, San Miguel de Tucuman, Argentina).

### Maintenance of lactic cultures and inoculum preparation

The strain of *Lactobacillus acidophilus* CRL 1014 was stored frozen (-80°C) in a medium composed of skim milk powder reconstituted at 10% and supplemented with 1.0% glucose and 0.5% yeast extract. The cells of *L. acidophilus* CRL 1014

were reactivated in MRS broth at a concentration of 10% (v/ v) and incubated at 37°C for 16 hours. The culture was centrifuged (3.000 x $g$/10 min) and the supernatant discarded. The cells were washed twice with phosphate-buffered water, before inoculation of the meat mixture. The population of *L. acidophilus* in the inoculum was at least $10^8$ CFU/ mL.

### Preparation of okara flour

Okara flour was obtained by drying the fresh soybean solid residue from the soymilk extraction process in an oven with forced air circulation, for about 8 hours at 60°C (Larosa *et al.*, 2006). After drying, the residue was cooled to room temperature and pulverized in a ball mill for 12 hours (Tecnal, Brazil). Then the flour was sieved at 30 mesh and stored at -18°C.

### Hamburger formulation and processing

In previous studies, this research group has assessed the physical and sensory properties of a chicken hamburger fermented with *Lactobacillus acidophilus* CRL 1014, containing 10 to 50% okara flour (Bomdespacho *et al.*, 2011). The results obtained indicated that only the formulation containing 10% okara showed appropriate characteristics and was well accepted by potential consumers, and this was chosen as the base formulation for this study (Table 1). Three treatments were performed on chicken hamburgers processed from 90% lean chicken meat and 10% okara flour as raw materials:
-Formulation F1: raw materials plus ingredients fermented with *Lactobacillus acidophilus* CRL 1014.
-Formulation F2: raw materials plus ingredients and 75 mg of sodium nitrite/kg, fermented with *Lactobacillus acidophilus* CRL 1014.
-Formulation F3: raw materials plus ingredients and 150 mg of sodium nitrite/kg, unfermented.

The amount of raw materials and other ingredients used in the processing of hamburgers was based on the formulation described in the literature for the corresponding beef product containing *okara* flour, with modifications (Table 1) (Turhan *et al.*, 2007).

Portions of skinned and lean chicken fillet were ground using a 16 mm disc. Then, the other ingredients were mixed with the meat (temperature between 6°C and 7°C) in the following order: ice, *okara* flour, seasonings (salt, pepper, fresh onion, dehydrated garlic, nutmeg), Simplesse®, BHT, cellulose and, when required, sodium nitrite and nitrate and dextrose. As the chicken fillets were fat-free, it was necessary to add fat replacer (Simplesse ®) to keep the characteristic texture of the hamburgers. Finally, the

Table 1. Raw materials and ingredients (%) used in the formulation of hamburgers

| Composition | Treatments* | | |
|---|---|---|---|
| | F1 | F2 | F3 |
| **Raw materials** | | | |
| Chicken meat | 90 | 90 | 90 |
| *Okara* | 10 | 10 | 10 |
| **Ingredients **** | | | |
| Ice | 20 | 20 | 20 |
| Pepper | 0.2 | 0.2 | 0.2 |
| Garlic powder | 0.2 | 0.2 | 0.2 |
| Raw anion | 2.0 | 2.0 | 2.0 |
| Nutmeg | 0.1 | 0.1 | 0.1 |
| Sodium Chloride | 1.5 | 1.5 | 1.5 |
| Dextrose | 1.5 | 1.5 | - |
| BHT | 0.01 | 0.01 | 0.01 |
| Simplesse® | 1.0 | 1.0 | 1.0 |
| Sodium Nitrite | - | 0.008 | 0.015 |
| Sodium Nitrate | - | 0.015 | 0.030 |
| Cellulose | 2.0 | 2.0 | 2.0 |
| LAB*** | 2.5 | 2.5 | |

F1 - 90% chicken meat and 10% *okara* flour, fermented with *L. acidophilus* CRL 1014; F2 - 90% chicken meat, 10% okara flour and 75 mg/kg sodium nitrite, fermented with *L. acidophilus* CRL1014; F3 - 90% of chicken meat, 10% *okara* flour and 150 mg/kg sodium nitrite, unfermented.
** Percent relative to the weight of raw materials. *** Lactic Acid Bacteria (*Lactobacillus acidophilus* CRL 1014)

probiotic starter culture of *Lactobacillus acidophilus* CRL 1014 was added to the formulations F1 and F2 in sufficient quantity to achieve $10^8$ CFU/ g in the final product and mixed to ensure its homogeneous distribution in the meat.

The resulting mixtures (F1 and F2) were placed in pots of high density polyethylene (HDPE), covered with PVC, and incubated in a BOD chamber, at a relative humidity of 80% and temperature around 10°C, until the pH fell to 5.1. The relative humidity of the chamber was controlled by an exposed saturated solution (70%) of ammonium sulfate.

The product obtained after the fermentation was cast into hamburger units of about 125 grams, diameter of 10 cm and height of 1 cm. Formulation F3 (unfermented) was maintained at -18°C. All products were pre-frozen (-10°C), packed in plastic bags of low-density polyethylene (LDPE), labelled and stored at a temperature of -18°C until analyzed.

For physical (shrinkage, yield, cooking) and sensory analysis, the hamburgers were grilled on hotplate (Edanca Prata, Brazil), previously heated for about 12 minutes at 160°C, being turned every two minutes until they reach a internal temperature of 75°C. The internal temperature was controlled with a digital thermometer equipped with a stainless steel thermocouple (OMH Delta, Italy) (Seabra *et al.*, 2002).

## Methods

### *Physical and chemical analysis*

#### *Cooking yield and percentage of shrinkage*
The cooking yield was assessed by weighing before and after cooking and the percentage of

shrinkage based on the variation of the diameter of the units (Seabra *et al.*, 2002). Eight replicates were used in each test.

### *Chemical composition*
The moisture, protein and ash were determined by methods approved by Association of Official Analytical Chemists (AOAC, 1995) and lipid concentrations by the method recommended by the Brazilian Ministry of Agriculture and Supply (MAPA, 1999). The total carbohydrates ware calculated by difference (Turhan *et al.*, 2007):

% Total carbohydrates = 100% - % (moisture + protein + lipid + ash).

### *Determination of pH*
pH was measured on a digital pH meter with glass electrodes (Qualxtron, USA) in samples prepared by mixing 10 g of hamburger and 10 mL of water (IAL, 2005). The pH was monitored it reached the value 5.1.

### *Microbiological analysis*
The products were analyzed microbiologically for the classes of microorganisms regulated by the National Agency of Sanitary Surveillance for hamburgers (ANVISA, 2001). For formulations F1 and F2, the samples were taken at the beginning and end of the fermentation step (day 6). Formulation F3 (unfermented) was kept under freezing and the samples for microbiological analysis were taken on days 0 and 6 of the storage period. The analysis of sulphite-reducing clostridia, *Salmonella* spp. and coagulase-positive staphylococci was performed by the methodology described by Downes and Ito (2001). For the total coliform count and *E. coli* test, the methods proposed by the Association of Official Analytical Chemists (AOAC, 2000) were used.

### *Sensory analysis*
The sensory tests, approved by the Ethics Committee of the FCF/UNESP, were performed in individual booths in the Sensory Analysis Laboratory. The hamburgers were grilled and served sliced for analysis at a temperature of approximately 45°C. The samples were presented to consumers in a randomized complete block design, monadically, on disposable white plates, labeled with three-digit numbers. The team consisted of 60 untrained consumers, recruited from students and staff of FCF - UNESP, Araraquara, Brazil, all accustomed to the consumption of hamburger made with chicken meat. In the acceptance test, the attributes of color, aroma, texture, flavor and overall impression were assessed,

Table 2. Physical parameters and chemical composition
of the raw hamburgers

| Physical parameters and chemical composition (%) | Treatment* | | |
|---|---|---|---|
| | F1 | F2 | F3 |
| Shrinkage | $9.00^a \pm 1.50$ | $8.00^a \pm 2.00$ | $5.10^b \pm 1.30$ |
| Cooking yield | $88.80^{a,b} \pm 1.90$ | $86.80^b \pm 4.30$ | $91.00^a \pm 2.40$ |
| moisture | $69.33^a \pm 0.55$ | $69.97^a \pm 1.59$ | $70.47^a \pm 2.04$ |
| Protein | $20.33^a \pm 1.23$ | $19.67^a \pm 1.3$ | $19.87^a \pm 1.20$ |
| fat | $3.90^a \pm 0.20$ | $3.67^a \pm 0.23$ | $3.67^a \pm 0.21$ |
| ash | $0.93^a \pm 0.06$ | $0.90^a \pm 0.10$ | $0.93^a \pm 0.06$ |
| Total carbohydrates | $5.50^a \pm 0.72$ | $5.80^a \pm 0.69$ | $5.07^a \pm 0.60$ |

\* F1 - 90% chicken meat and 10% okara flour fermented with *L. acidophilus* CRL 1014;   F2 - 90%  chicken meat, 10% *okara* flour and 75 mg/kg nitrite, fermented with *L. acidophilus* CRL1014;  F3 - 90%  chicken meat, 10% *okara* flour and 150 mg/kg nitrite, unfermented. Means with same letter in the same row do not differ significantly (p < 0.05). All tests were performed in triplicate

using a structured hedonic scale of nine points (Stone and Sidel, 1993). Intention to purchase was assessed on five-point category scale, ranging from "definitely would buy" to "certainly would not buy" the product (Meilgaard, Civille, Carr, 1999).

*Statistical analysis*

The data were subjected to analysis of variance (ANOVA) and the Tukey test, adopting a significance level of 5%. Statistical analysis was performed with BioStat software.

**Results and Discussions**

Table 2 presents the mean values of physical parameters and chemical composition of the raw hamburgers.

*Coking yield and shrinkage*

With regard to cooking yield, while treatment F3 led to the largest mean yield, this did not differ significantly from treatment F1. There was also no statistical difference (p < 0.05) between treatments F1 and F2 (fermented products). The fermented hamburgers (F1 and F2) showed higher values for the shrinkage parameter than F3 (p < 0.05), indicating that the fermentation contributed significantly to reducing the diameter of the products.

The water-holding capacity is a crucial property for the quality of meat and may be defined as the capacity of the meat to retain moisture during the application of external process such as cutting, grinding and heating. When the water-holding capacity falls, a loss of moisture and weight reduction of the product is observed during cooking (Prandl *et al*., 1994).

This functional property of meat is lowest at a pH between 5.2 and 5.3, the isoelectric point of most of the muscle proteins. Thus, at the end of the fermentation process, the production of acid and the drop in pH result in a decreased ability to retain water, explaining the reduced yields after cooking of
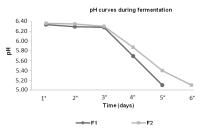


Figure 1. Variation in pH during fermentation of hamburgers with *Lactobacillus acidophilus* CRL 1014.

F1 - 90% chicken meat and 10% *okara* flour fermented with *L. acidophilus* CRL 1014; F2 - 90% chicken meat, 10% *okara* flour and 75 mg/kg nitrite, fermented with *L. acidophilus* CRL1014. All tests were performed in triplicate.

the fermented products.

*Chemical composition*

The Ministry of Agriculture (Brazil) recommends that hamburgers meet the following requirements in relation to chemical composition: fat (maximum) 23.0%, protein (minimum) 15.0%, total carbohydrates 3.0%, calcium content (dry basis maximum) 0.1% in crude product  and 0.45% in cooked product (MAPA, 2000). As can be seen in Table 2, there was no significant difference between samples (p < 0.05) in relation to chemical constituents. The concentrations of lipids and proteins in all samples are within the limits required by Brazilian legislation, detailed in the Technical Regulation for Identity and Quality of hamburgers (MAPA, 2000).

In other studies that used okara flour in the formulation of hamburgers, there is a wide variation in the measured chemical composition, probably due to the nature of the raw materials used. In a study who analyzed a bovine raw meat product with 10% okara added, it was found as follows: 58.89% moisture, 20.43% protein, 14.01% of lipids, 2.84% ash and 3.84% carbohydrates (Turhan *et al.*, 2009).

Another study conducted with chicken hamburger reported the following chemical composition: 20.65% of protein; 6.57% of lipids and 1.4% carbohydrates (Leonardi *et al.*, 2009). The low level of lipids found in hamburgers in the present study was mainly due to use of the chicken fillet, without skin or fat. In addition, the *okara* composition (37.5% protein; 32.1% carbohydrates; 11.9% fat; 15.5% fibers and 3.0% ash) probably contributed to the higher concentration of carbohydrates found in this study.

*Measurement of pH*

The decline of the final pH to below 5.3 during the first days of fermentation is important to ensure the quality and safety of fermented products, by giving desirable sensory characteristics and inhibiting the growth of pathogens (Leistner, 1990; Lücke, 2000). The changes in pH during the manufacture of fermented hamburgers (F1, F2) are shown in Figure

Table 3. Microbiological analysis of different raw hamburgers

| Groups of Microrganisms | Treatments* | | | | | |
| | F1 | | F2 | | F3 | |
| (log$_{10}$CFU/g) | T1 | T2 | T1 | T2 | T1 | T2 |
| --- | --- | --- | --- | --- | --- | --- |
| Coagulase-positive staphylococci | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 |
| Sulphite-reducing clostridia | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 |
| *Salmonella* spp. (Abs./Pres.) | Abs. | Abs. | Abs. | Abs. | Abs. | Abs. |
| Total Coliforms | 2.28 | 4.78 | 2.48 | 3.08 | 2.54 | 2.30 |
| *E. coli* | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 | <1.0 |

T1: Initial time - for F1 and F2 this corresponds to the period prior to fermentation; for F3, the period prior to freezing storage.
T2: Final time - for F1 and F2, after fermentation (six days after preparation); for F3, six days after preparation under freezing storage.
* F1- 90% chicken meat and 10% *okara* flour fermented with *L. acidophilus* CRL 1014; F2 - 90% chicken meat, 10% *okara* flour and 75 mg/kg nitrite, fermented with *L. acidophilus* CRL1014; F3 - 90% chicken meat, 10% *okara* flour and 150 mg/kg nitrite, unfermented.
All tests performed in triplicate; Abs.: Absent in 25 grams.

1. During the first six days of fermentation, there was a fall in pH from 6.33 to about 5.1, as a consequence of the accumulation of lactic acid produced by *Lactobacillus acidophilus* CRL 1014 during the fermentation process. Similar results were obtained by Sawitzki (2000) who studied the use of lactic acid bacteria in the production of Italian salami. The authors found a reduction in pH values from 5.89 to 5.14 after one week of fermentation.

The development of fermented meat products requires the use of bacteria resistant to sodium nitrite, nitrate and sodium chloride and able to multiply rapidly during fermentation (Papamanoli *et al.*, 2003). Sameshima *et al.* (1998) tested the ability of 202 species of probiotic lactobacilli of intestinal origin to resist sodium nitrite and sodium chloride added to a liquid medium and found that the strains *L. paracasei* ssp. *paracasei* FERM P-15121, *L. rhamnosus* PERM P-15120 and *L. acidophilus* FERM P-15119 were tolerant to these salts. In this study, the fermented product processed without the addition of curing salts (F1) showed faster acidification, indicating that the presence of sodium nitrite and nitrate may delay the propagation of the starter culture (*Lactobacillus acidophilus* CRL 1014). However, the increase in total fermentation time was only one day, which was not detrimental to the process.

*Microbiological analysis*

The poultry sent for slaughter is usually the initial source of contamination in chicken products and the number of microorganisms can be influenced by the hygienic conditions of slaughter and processing (Lírio *et al.*, 1998). The microbiological results of the different products prior to and after fermentation are given in Table 3.

Brazilian Sanitary Regulation 12/2001 (ANVISA, 2001) defined the microbiological limits for meat products, including hamburgers, as a maximum of 5x10$^3$ CFU/g for coagulase-positive staphylococci,

3x10$^3$ CFU/g for sulphite-reducing clostridia, 5x10$^3$ CFU/g for fecal coliform and the absence of *Salmonella* spp.

Although the legislation does not determine limits for microorganisms belonging to the total coliform group, this analysis was included because it reflects the hygienic handling of the product, being indicative of contamination due to failure during processing, improper cleaning or insufficient heat treatment (Pardi *et al.*, 1993). Analysis of *E. coli* is justified by the fact that this microorganism is the main component of the fecal coliform group and associated with some pathogenic strains of this species.

According to the microbiological standards established by Brazilian law, all hamburgers processed in this study were suitable for consumption; there was no variation between times or treatments in the populations of microorganisms belonging to the groups analyzed, except for total coliforms. For this group, there was an increase of two logarithmic cycles for fermented hamburger without addition of any curing salts (F1) and one log cycle for fermented hamburger with a 50% of reduction in curing salts (F2), demonstrating that the fermentation alone was not able to inhibit the growth of these microorganisms. This was to be expected, since the coliforms are tolerant to acidic media and somewhat sensitive to the presence of curing salts (Sacco Brasil, 2005). It is noteworthy that sodium nitrite acts as a bacteriostatic agent in acidic medium for anaerobic microorganisms (Frazier and Westihoff, 2008).

The lactic acid produced by the culture starters during fermentation causes a reduction in pH, resulting in a change in homeostasis and inhibition of the proliferation of different pathogens (*Staphylococcus aureus*, *Clostridium* spp., *Salmonella* spp.) and spoilage organisms (*Pseudomonas* spp.) (Jay, 2005). In addition, some culture starters are capable of producing bacteriocin and other antibacterial compounds that aid in preserving of the food product (Dicks *et al.*, 2004; Muthukumarasamy and Holley, 2007). In the literature there are several studies that aim to demonstrate the efficacy of probiotic cultures in the preservation of meat products, all showing positive results (Dicks *et al.*, 2004; Muthukumarasamy and Holley, 2007; Pidcock *et al.*, 2002).

The sodium nitrite added to meat products is particularly important at the start of the fermentation, to inhibit the growth of microorganisms, such as *Clostridium* spp., since the pH of the mixture has not yet been sufficiently reduced. Nitrite in the form of nitrous acid (HNO$_2$) is capable of penetrating the barrier of the bacterial cell wall and altering its

Table 4. Mean values (± SD) of sensory attributes
assessed by the consumers

| Treatments* | Means | | | | |
|---|---|---|---|---|---|
| | Color | Odor | Texture | Flavor | Overall impression |
| F1 | 7.0 [a] ±1.6 | 7.2 [a] ±1.4 | 6.6 [b] ±1,8 | 7.1 [a] ±1,6 | 6.9 [a] ±1.5 |
| F2 | 7.4 [a] ±1.4 | 7.1 [a] ±1.5 | 6.6 [b] ±1.9 | 7.0 [a] ±1.4 | 7.1 [a] ±1.3 |
| F3 | 7.5 [a] ±1.2 | 7.0 [a] ±1.6 | 7.6 [a] ±1.2 | 7.0 [a] ±1.2 | 7.4 [a] ±1.1 |

Different letters in the same column indicate significant difference between treatments for
the Tukey test (p < 0.05). n = 60 consumers.
* F1 - 90% chicken meat and 10% *okara* flour fermented with *L. acidophilus* CRL 1014; F2
- 90% chicken meat, 10% *okara* flour and 75 mg/kg nitrite, fermented with *L. acidophilus*
CRL1014; F3 - 90% chicken meat, 10% *okara* flour and 150 mg/kg nitrite, unfermented.

metabolism, thereby preventing the development of undesirable microorganisms.

So, based on the results of this study, it can be stated that the fermentation was able to inhibit the multiplication of microorganisms of the groups of coagulase-positive staphylococci, sulphite-reducing clostridia, *Salmonella* spp. and *E. coli*, in treatments with a reduction in the curing salts concentration and even without the addition of any of this preservative. On the other hand, fermentation alone was not sufficient to control the growth of the total coliforms group.

### Sensory analysis

#### Acceptance of the products

The ideal probiotic cultures for use in meat-based fermented foods are those that do not interfere negatively in the technological and sensory properties of the products (Ammor and Mayo, 2007). Several studies have demonstrated that the probiotic cultures of *Lactobacillus casei*, *Lactobacillus acidophilus* and *Bifidobacterium lactis* not affect the flavor and aroma characteristics of fermented meat products (Andersen, 1998; Pidcock *et al*., 2002; Ammor and Mayo, 2007).

The three different treatments were subjected to an acceptance analysis and the results are shown in Table 4. The hamburgers had high scores for all attributes, without differing among themselves in relation to color, aroma, flavor and overall impression (p < 0.05). On the other hand, the unfermented product with the addition of curing salts (F3) exhibited a greater acceptance for texture, differing significantly from the other processes (F1 and F2, p < 0.05). This result suggests that the fermentation process changes the texture of hamburgers, probably due to the reduction in pH and consequent decrease in the water retention capacity after cooking.

Macedo *et al.* (2008) analyzed sausages processed with the probiotic cultures of *Lactobacillus casei*, *Lactobacillus paracasei* ssp. *paracasei* and *Lactobacillus casei* ssp. *rhamnosus*. The product to which *Lactobacillus paracasei* was added showed sensory characteristics greatly appreciated by the

consumers, with the highest scores for texture and color. The product also had a pronounced acid flavor, confirmed by measurements of pH and acidity.

The factors that contribute to the sensory characteristics of fermented products include: type of raw material, spices, starter culture and salt curing. Lactic acid and the products resulting from the action of proteolytic and lipolytic enzymes are primarily responsible for the characteristic flavor of fermented meat products (Sebranek and Bacus, 2007).

Regarding the use of okara flour in meat products, Turhan *et al*. (2009) found that the acceptance of the samples decreased significantly (p < 0.05) when adding okara flour was greater than 7.5%. In another study was prepared goat meat hamburger with reduced fat content, with 0%, 15%, 20% and 25% *okara*, in the wet form. The results showed that the emulsion stability decreased with increasing content of okara. The hamburgers with 15% *okara* had higher acceptance for flavor, juiciness and overall acceptability than the control. The authors concluded that the *okara* can be used to replace meat in concentrations up to 15% in goat meat hamburgers without affecting the sensory quality and acceptability of products (Das *et al*., 2007). In this study, the concentration of *okara* flour did not vary among the different formulations, and thus did not interfere in the lower acceptance of the fermented products with respect to texture.

The purchasing intention test revealed that to the product containing 90% chicken, 10% *okara* flour and fermented with *L. acidophilus* CRL 1014 (F1), the majority of the consumers (66%) said that they would "probably buy" (48%), "certainly would buy" (18%) or had doubts about the purchase of the product (19%). In the formulation containing 90% chicken, 10% okara flour, 75 mg/kg of nitrite and fermented with *L. acidophilus* CRL 1014 (F2), the majority of the consumers (72%) said they would "probably buy" (45%) or "certainly would buy" the product (27%). The formulation containing 90% chicken, 10% *okara* flour, 150 mg/kg of nitrite and non-fermented (F3) showed a greater purchasing intention than the other samples, with about 80% of consumers responding they would "probably would buy" or "certainly would buy" the product.

Among the possible reasons for the better performance of the non-fermented sample (F3) in relation to intent to buy are: the texture of the product, seen to score best in the acceptance test, and the fact that the product is similar to a conventional chicken hamburger commercially available. It should be noted that although the consumers are not accustomed to consumption of fermented hamburger,

the formulations processed with *Lactobacillus acidophilus* CRL 1014 showed suitable sensory characteristics, with potential chances of being industrialized.

## Conclusion

This study demonstrated that fermentation with *Lactobacillus acidophilus* CRL1014 as the starter culture was effective in maintaining the safety of the hamburger processed with chicken meat and okara flour, even with partial or total reduction of curing salts (nitrite and nitrate). The fermented hamburgers exhibited appropriate sensory properties, being well accepted by potential consumers. Additional studies are necessary to investigate the metabolites produced during the fermentation process that are probably related to safety and quality of the products.

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