Lactobacillus alimentarius UTILIZATION FOR SAUSAGE BIOPRESERVATION

Liana Ines Guidolin Milani; Lucia Schuch Boeira; Lisiane Soriano Bessa; Viviane Melo;Nelcindo Nascimento Terra
Departamento de Tecnologia e Ciencias dos Alimentos, Universidade Federal de Santa Maria, 97.119-900 - Santa Maria -RS - Brasil.

INTRODUCTION

Meat can be highly perishable when improperly refrigerated due to its high water activity, its pH close to neutrality and the great quantity of nutrients (FRAZIER, 1993). Meat industrialisation lengthen meat shelf-life, as in sausage, which can be preserved for a long time under refrigeration. In the industrialisation process, there is a potential risk of incorporating bacteria to the sausage surface, during packing, which can cause alterations capable of shorten the shelf-life the product (SARANTOPOULOS, 1992).

Recently, it has been emphasised the antimicrobial effects of lactic acid bacteria for meat products biopreservation. These antimicrobial effects are probably due to a synergistic effect of the various antimicrobial properties of the lactic acid bacteria as the production of organic acids, peroxide, carbon dioxide, the lowering of the oxidation-reduction potential, nutrient competition and bacteriocin production (ANDERSEN, 1995; JACK et alli, 1995; PIARD e DESMAZEUD, 1992; KLAENHAMMER, 1988).

The major mechanism of action of *Lactobacillus alimentarius is* through nutrient competition with bacteria that causes product alterations as spoilage. Therefore, it is recommended the addition of high levels of *Lactobacillus alimentarius* in the inocullum (107 CFU/g), to ensure a high level of initial activity (ARMSTRONG, 1996).

The fermentation of glucose and sucrose by *Lactobacillus alimentarius* produces very low acidity which has many advantages as there is no effect on flavor and taste of treated samples, due to the limited lipolytic and proteolytic activity of these bacterial group (ARMSTRONG, 1996).

Lactobacillus alimentarius was tested in various meat product as: sliced ham, ground beef, Frankfurter sausage, fresh meat wrapped by vacuum and bacon (ARMSTRONG, 1996; LAULUND, 1996).

ANDERSEN (1995) demonstrated that *Lactobacillus alimentarius* was capable of inhibiting *Listeria monocytogenes* in Frankfurter sausage and bacon cubes without altering either the pH or the censorial characteristics of the samples. Total quality product was maintained during storage because *Lactobacillus alimentarius* was capable of inhibiting microorganisms that produces slime or bad smell.

The objective of this work was to evaluate *Lactobacillus alimentarius* performance on Frankfurter sausage. The samples were stored at 5°C and 10°C based on the fact that during sausage transport and storage, there are possibilities of variations on recommended refrigeration temperatures.

MATERIAL AND METHODS

SAMPLES In this experiment it was used Frankfurter sausage which presented the following contents: beef, poultry meat (mechanically separated), *curing salts,* polyphosphates, starch, spices and salt.

***Lactobacillus alimentarius* INOCULATION** A suspension of *Lactobacillus alimentarius,* consisting of 100 ml of destined water and 50g of FLORA CARN L2 (Christhen Hansen Valinhus S.A.), was used to inoculate sausage samples. The inocullum suspension was sprayed on the sausage surface resulting in approximately 107 CFU/cm2 of *Lactobacillus alimentarius.*

SAMPLE PACKAGING AND STORING After spray application of the *Lactobacillus alimentarius* suspension, the sausages were vacuum wrapped in packages weighing approximately 250g and stored for 8 weeks at 5°C (+- 0,1 °C) and 10°C (+- 0,1 °C) separately.

MICROBIOLOGICAL ANALYSES The sausages were analyzed right after treatment and every week. SWAB tests were done on sausage surface at three sampling points resulting in an area of evaluation of 12 cm2. The swab was mixed with 12 ml of peptonated water, and this dilution was considered the zero dilution, which was used to do the other dilution's. The dilution's were platted all microbiological analyses. Total count for aerobic mesophillic and psicrophillic microorganisms were done using standard agar (MERCK), incubated at 32°C for 48 hours and at 7°C for 8 days, respectively. Lactic acid bacteria counts were done using MRS agar with double layer and were incubated at 32°C for 5 days.

Also, evaluation of Gram negative microorganisms were done utilising standard agar for counting (MERCK) and by adding 1 ppm of crystal violet and 50 ppm of 2-3-5- triphenyl tetrazolium cloride and incubating at +/- 32°C for 48 hours (GILLILAND AND SPECK, 1975).

WATER ACTIVITY The water activity determination was done after spray application following TERRA and BRUM (1988).

pH pH determination was carried out using a DIGIMED potentiometer equipped with a glass electrode, at the same time as the microbiological analysis (TERRA and BRUM; 1988).

SENSORIAL ANALYSIS Simultaneously to the microbiological analysis, the sausages were evaluated by a panel of tasters that graded the product in a scale from 0 to 10 for product color, flavor, taste, texture and appearance. Grades ranging from 9 to 10 were considered very good, from 7 to 8.9 good, from 5 to 6.9 regular, from 3 to 4.9 bad and from 0 to 2.9 unacceptable. Sausages were warmed up for 5 minutes before censorial analysis started.

RESULTS AND DISCUSSION

The positive effects of *Lactobacillus alimentarius* utilisation on carcass biopreservation was observed in the control of Gram negative microorganisms, on samples treated with *Lactobacillus alimentarius* and maintained at 5°C. Reductions of two logarithmic cycles for Gram negative microorganisms, were obtained when compared with controls, by the 6th week of storage. At the same time, when samples treated with *Lactobacillus alimentarius* were maintained at 10°C, a reduction of one logarithmic cycle was observed (Figure 1).

Samples treated with *Lactobacillus alimentarius* showed increased aerobic mesophyllic and psicrotrophic microorganisms counts at the beginning (106 CFU/cm~2) due to the growth of the inoculated lactic acid bacteria. In contrast, the control samples presented very low initial counts (approximately 102 CFU/cm~2), increasing the number of microorganisms during storage, ultrapassing the counts of treated samples. LAULUND (1996) suggests that total meat quality and meat products quality is influenced not only by total cells counts, but also by bacterial flora composition.

Lactic acid bacteria growth can be observed on Figure 4, in which samples inoculated with *Lactobacillus alimentarius* presented higher levels of lactic bacteria right after inoculation (approximately 107 CFU/cm2) compared to the controls (approximately 10' CFU/cm2). However, lactic acid bacteria counts in control samples increased during storage reaching the same number as inoculated ones. A high level of bacteria in the inoculum at the beginning, its very important, since it increases the levels of initial activity, ensuring the inhibitory mechanism of nutrient competition (ARMSTRONG, 1996).

SARANTOPOULOS (1992) demonstrated that inside vacuum packages, "indigenous" lactic acid bacteria, heterofermentative, that produce gases can develop. In our study, during storage, it was observed the development of a white liquid on the surface of control samples as well as gas production within the packages, which contributed to the destruction of the vacuum. These alterations were observed in control samples stored at 10°C after the 28th day, and for samples stored at 5°C after 35th day of storage. The sausages inoculated with *Lactobacillus alimentarius* and stored at 10°C also showed these alterations, during the same time period, but with lower intensity than the control samples. On the other hand, samples inoculated with *Lactobacillus alimentarius* and stored at 5°C, did not show these alterations and had a very good appearance. The results of this experiment agree with ARMSTRONG (1996), which concluded that Starter cultures are capable of suppressing these alterations.

In average, the grades attributed to the censorial properties of sausage samples treated with *Lactobacillus alimentarius* were slightly lower than controls at the beginning of the experiment. However, after some time, the grade average increased when compared to the controls, indicating that *Lactobacillus alimentarius* contributed to the maintenance of the general qualities of the samples during the storage period.

Samples treated with *Lactobacillus alimentarius* and kept at 10°C presented a rapid drop in pH by the 7th day (Figure 5), while the controls gradually lowered the pH during storage.

Sausage inoculation with *Lactobacillus alimentarius* did not affect its water activity, since all samples presented a value of 0,97 for water activity after the treatment.

CONCLUSION

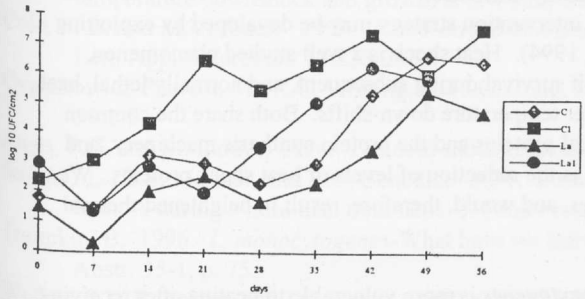
Based on the results, we can conclude that the culture of *Lactobacillus alimentarius* was effective in controlling undesirable microorganisms in vacuum packages, being more effective when kept at 5°C. Under abusive temperature (10°C), the culture assured a better general product quality, but was

not as effective as under 5°C.

LITERATURE CITED

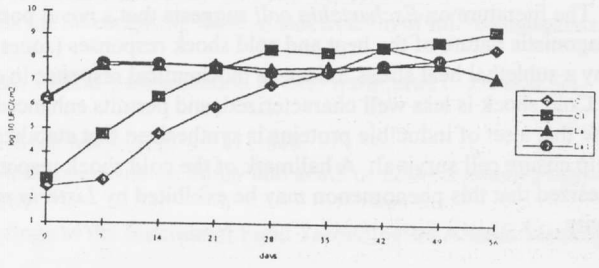
01. ANDERSEN, L. Biopreservation with flora carn L-2. Fleischwirtsch, 75 (11), 1327-29, 1995.
02. ARMSTRONG, H. Competitive culture for fresch meat. Meat international - The world wide manazine on meat trade and technolo~Y, V.5 (3), 14-17, 1996.
03. FRAZIER, W.C.; WESTHOFF, D.C. Microbiologia de los alimentos. 4a edi,cao. Editora Acribia, 1993, 681 p.
04. GILLILAND, S.E. and SPECK, M.L. Inibition of psychrotrophic bacteria by lactobacilli and pediococci in non fermented refrigerated foods. Journal of food science. 40: 903-905, 1975.
05. JACK, R.N.; TAGG, J.R.; RAY, B. Bacteriocins of Gram positive bacteria. Microbiolonical reviews. V. 59 (2), 171-200, 1995.
06. KLAENHAMEER, T.R. Bacteriocins of lactic acid bacteria. Biochimie. V. 70, 337-349, 1998.
07. LAULUND, E. Strategies for procuring natural ingredients for the food industry. Danish dairy food industrv...world wide, (10), 59-61, 1996.
08. PIARD, J.C.; DESMAZEUD, M. Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antibacterial substances. Lait. V. 72, 113-142, 1992.
09. SARANTOPOULOS, C.I.G.L.; PASSOS, R.B.; DESTRO, M.I.; SHIROSE, I. Estudo da estabilidade de salsicha embalada a vacuo e pasteurizada. Revista Nacional da Came. Ano XVII (190), 41-47, dezembro 1992.
10. TERRA, N.N.; BRUM, M.A.R. Carne e seus derivados: tecnicas de controle de qualidade. Sao Paulo: Nobel, 1988. 121 p.

Figure 1. Development of Gram negative bacteria (log of CFU/cm2) on sausages during storage at 5°C (+/- 0.1°C) and at 10°C (+/- 0.1°C).



c= Control sausages stored at 5°C
cl= Control sausages stored at 10°C
La= Sausages inoculated with *Lactobacillus alimentarius* stored at 5°C
Lal= Sausages inoculated with *Lactobacillus alimentarius* stored at 10°C

Figure 2. Development of aerobic mesophyllic microorganisms (log of CFU/cm2) in sausages during the storage period at 5°C (+/- 0.1°C) and at 10°C (+/- 0.1°C).



c= Control sausages stored at 5°C
cl= Control sausages stored at 10°C
La= Sausages inoculated with *Lactobacillus alimentarius* stored at 5°C
Lal= Sausages inoculated with *Lactobacillus alimentarius* stored at 10°C

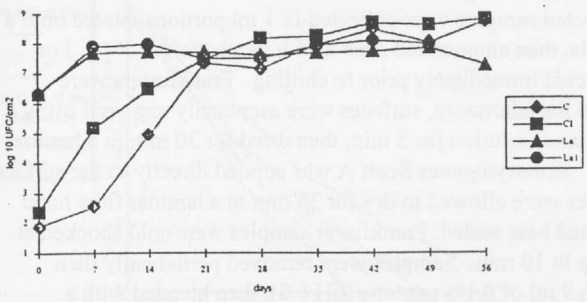
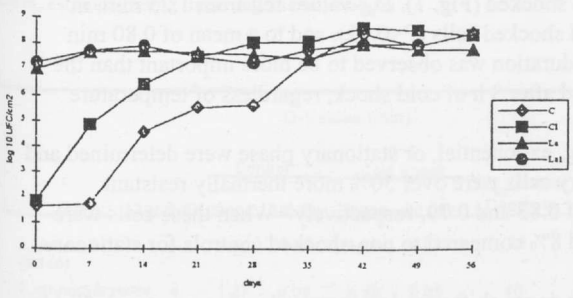


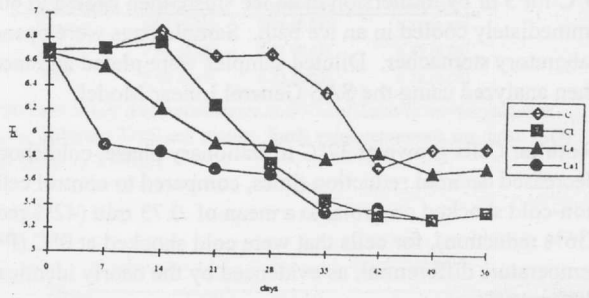
Figure 3. Development of psichrotrophic microorganisms (log of CFU/cm2) in sausages during the storage period at 5°C (+/- 0.1°C) and at 10°C (+/- 0.1°C).

c= Control sausages stored at 5°C
cl= Control sausages stored at 10°C
La= Sausages inoculated with *Lactobacillus alimentarius* stored at 5°C
Lal= Sausages inoculated with *Lactobacillus alimentarius* stored at 10°C



c= Control sausages stored at 5°C
cl= Control sausages stored at 10°C
La= Sausages inoculated with *Lactobacillus alimentarius* stored at 5°C
Lal= Sausages inoculated with *Lactobacillus alimentarius* stored at 10°C

Figure 4. Development of Lactic acid bacteria (log of CFU/cm2) in sausages during the storage period at 5°C (+/- 0.1°C) and at 10°C (+/- 0.1°C).



c= Control sausages stored at 5°C
cl= Control sausages stored at 10°C
La= Sausages inoculated with *Lactobacillus alimentarius* stored at 5°C
Lal= Sausages inoculated with *Lactobacillus alimentarius* stored at 10°C

Figure 5. Sausage pH during the storage period at 5°C (+/- 0.1°C) and at 10°C (+/- 0.1°C).