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Original

# Microbiota of sausages obtained by spontaneous fermentation produced in the South of Brazil

*Microbiota de salames obtidos por fermentação espontânea produzidos no Sul do Brasil*

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**Abstract**

The study of the ecology of fermented sausage is fundamental to understand the physical and chemical changes that happen during fermentation and maturation. The aim of the present study was to determine the microbiological characteristics of sausages produced by spontaneous fermentation. Fifty samples of sausages produced in the South of Brazil by different small manufacturers were analyzed for the following microbiota: aerobic mesophilic bacteria; *Micrococcaceae*; mold and yeast; lactic acid bacteria; total and fecal coliforms; coagulase-positive *Staphylococcus*, and *Salmonella*. In most samples (72%), the count of lactic bacteria was higher than 6 $\log_{10}$ cfu.g$^{-1}$, and the samples with the highest counts were above 8 $\log_{10}$ cfu.g$^{-1}$. The counts of *Micrococcaceae* in most samples were between 5 $\log_{10}$ and 7 $\log_{10}$ cfu.g$^{-1}$. With respect to the presence of molds and yeasts, there was a significant variation among the samples with counts ranging from 2 $\log_{10}$ cfu.g$^{-1}$ and 6 $\log_{10}$ cfu.g$^{-1}$. From the data obtained, it was possible to conclude that 24% of the analyzed samples did not comply with the current law in Brazil since the levels of fecal coliforms or coagulase-positive *Staphylococcus* exceeded the maximum limit allowed.
***Keywords:*** *sausage; microbiological characteristics; microbiological quality.*

**Resumo**

O estudo da ecologia de salames fermentados faz-se necessário para entender as mudanças físicas e químicas que ocorrem durante a fermentação e maturação. O objetivo deste trabalho foi determinar as características microbiológicas de salames produzidos por fermentação espontânea. Foram analisadas cinquenta amostras de salames de diferentes pequenas indústrias em relação à seguinte microbiota: bactérias aeróbias mesófilas; *Micrococcaceae*; bolores e leveduras; bactérias lácticas; coliformes totais e fecais; *Staphylococcus* coagulase positiva; e *Salmonella*. Na grande maioria das amostras (72%), a presença de bactérias lácticas foi superior a $10^6$ ufc.g$^{-1}$, sendo que as amostras com as maiores contagens tiveram quantidades acima de $10^8$ ufc.g$^{-1}$. A concentração de *Micrococcaceae*, na maioria das amostras, ficou entre $10^5$ e $10^7$ ufc.g$^{-1}$. Em relação à concentração de bolores e leveduras, houve uma grande variação entre as amostras, com quantidades próximas a $10^2$ ufc.g$^{-1}$ até $10^6$ ufc.g$^{-1}$. Pelos dados obtidos, concluiu-se que, das amostras analisadas, 24% estão em desacordo com as normas vigentes no Brasil, pois apresentam quantidades superiores do máximo permitido para coliformes a 45 °C ou *Staphylococcus* coagulase positiva.
***Palavras-chave:*** *salame; características microbiológicas; qualidade microbiológica.*

## 1 Introduction

A great variety of fermented sausages is produced and consumed in large quantities in Brazil. These products are made by both many small manufacturers as well as large companies in the meat industry. Among the fermented sausages produced, the colonial sausage, usually called 'salami', stands out. The Technical Regulation of Quality and Identity Standards defines sausage as an industrialized meat product made of pork and beef or pork alone, which is added with pork fat and ingredients, stuffed, cured, fermented, smoked or not, and dried (BRASIL, 2000a). Colonial sausages (salami), on the other hand, must be made only of pork (BRASIL, 2000b).

The manufacturing of many fermented products is based on the skill and experience of the meat manufacturer and not on scientific and technological knowledge. The fermentation of meat products is a complex biological process accelerated by the metabolic activity of desirable microorganisms in the presence of a large variety of competitors or species with synergistic action (BUCKENHÜSKES, 1993; SAMELIS et al., 1998).

Lactic acid bacteria are essential in the manufacturing process of different sausages. The ability of these bacteria to lower the pH of the mixture by producing lactic acid from sugars causes the development of desirable sensorial characteristics and inhibit the growth of pathogenic and spoilage bacteria providing more stable and safer final products (SAMELIS et al., 1994; HUGAS; MONFORT, 1997; TERRA, 1998; OLIVEIRA; MENDONÇA, 2004).

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Besides the lactic acid bacteria, representatives of the *Micrococcaceae* family are commonly found in sausages. Species of the genus *Staphylococcus*, such as *S. carnosus* and *S. xilosus*, are usually accounted responsible for color formation and stability due to the action of nitrate reductase and catalase enzymes. The activity of these microorganisms also contributes to the formation of flavor, reduces production time, prevents rancidity, and reduces the development of deteriorative microorganisms (PAPAMANOLI et al., 2002; SANZ et al., 1997).

The microbiologic stability of fermented sausages is determined by low water activity and a maximum pH of 5.0. The production of inhibiting substances by lactic acid bacteria and by *Micrococcaceae* also contributes to their microbiological stability and safety (LÜCKE, 2000; PAPAMANOLI et al., 2002). However, the hurdles in the microbial development in sausages may not be enough to avoid the survival and multiplication of some pathogens (TYÖPPÖNEN et al., 2003).

The microbiological quality of a product offers information that allows for the evaluation of its processing, storing and distribution for consumption, shelf life, and risks to the population's health (FRANCO et al., 2002; NASSU; GONSALVEZ; BESERRA, 2002). Important pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* have been isolated from colonial sausages and accounted responsible for outbreaks of foodborne disease (INCZE, 1998; PEREIRA, 2004; MOORE, 2004).

The aim of this study was to investigate the microbiota of sausages obtained by spontaneous fermentation and produced by small manufacturers in the South of Brazil.

## 2 Materials and methods

### *2.1 Samples*

Samples of sausages traditionally produced by spontaneous fermentation and manufactured by many different small sized companies operating in the meat industry in the South of Brazil were analyzed. The samples were purchased from street markets and supermarkets, taken to the laboratory, and stored under refrigeration until analyses.

### *2.2 Microbiological analysis*

For the microbiological analysis, the sausage casing was removed aseptically. Twenty-five grams were collected from each sample were mixed with 225 mL of sterile peptone water 0.1 (v/w) and homogenized for two minutes. The necessary decimal dilutions were made in the same diluent, and aliquots of the adequate dilutions were inoculated in duplicates in different culture media. The total count of aerobic mesophilic bacteria was done on plates with Plate Count Agar (PCA) after incubation for 48 hours at 35 °C; molds and yeasts were counted on plates with Dichloran Rose Bengal Chloramphenicol agar (DRBC) after incubation for 5 days at 25 °C; lactic acid bacteria on plates with De Man, Rogosa and Sharpe Agar (MRSA) after incubation for 48 hours at 35 °C; *Micrococcaceae* on plates with Mannitol Salt Agar (MSA) after incubation for 48 hours at 35 °C; and coagulase-positive *Staphylococcus* on plates with Baird Parker agar (BP) after incubation for 48 hours at 35 °C. The typical colonies were submitted to the coagulase test using the Staphclin system (LABORCLIN – Lab Products® Br); total coliforms (multiple tubes technique) in Bile Brilliant Green broth at 2% (VB) and were incubated at 35 °C for 24-48 hours; fecal coliforms (multiple tubes technique) in *Escherichia coli* broth with 4-methylumbelliferyl-β-D-glucuronide (EC - MUG) and incubated at for 24 hours at 44.5 °C; *Escherichia coli* (multiple tubes technique) using the positive tubes from the fecal coliform test; those with a phosphorescent culture in a dark chamber with an ultraviolet lamp (365 nm) were considered positive for *E. coli*.

In order to determine the presence of S*almonella* spp., pre-enrichment was carried out by adding 25 g of the sample to 225 mL of Lactose Broth and incubating at 35 °C for 18-20 hours. From the selective enrichment, 1 mL was transferred to tubes containing 10 mL of Tetrathionate Broth (TT) and Selenite-Cistine Broth (SC), and after being incubated for 24 hours at 35 °C, the cultures were striated on plates with Xylose Lysine Deoxychocolate agar (XLD), Bismuth Sulfite (BS), and Hektoen Enteric (HE). After this period of incubation, the characteristic colonies of *Salmonella* were biochemically identified by the BACTRAY I and II system (LABORCLIN – Lab Products® Br).

## 3 Results and discussion

The microbiological quality of the raw material and the presence of a native microflora capable of transforming the raw material into the final product are fundamental factors to produce good-quality sausage. In Brazil, a large majority of small manufacturers produce sausages by spontaneous fermentation. Therefore, fundamental factors to obtain good-quality sausages using this method are the microbiological quality of the raw material and the presence of a native microflora capable of transforming the raw material into the final product.

The study of the ecology of fermented sausages is fundamental to understand the physical and chemical changes that happen during fermentation and maturation. The microflora that develops in sausages is responsible for defining the final product's characteristics. Studies on sausages obtained by fermentation have been carried out in typical products from Greece, Spain, and Italy. Those studies aim at identifying and quantifying the microbial population that develops during the different stages in the production or in the final product, as well as identifying isolates of lactic bacteria, non pathogenic *Staphylococcus*, and molds and yeasts in sausages produced by spontaneous fermentation and selecting strains with adequate metabolic and physiological characteristics to be used as starters (SANZ et al., 1997; SAMELIS et al., 1998; SANTOS et al., 1998; COPPOLA et al., 2000; PAPAMANOLI et al., 2002; AMBROSIADIS et al., 2004; MORETTI et al., 2004; COMI et al., 2005).

### *3.1 Total mesophilic aerobic bacteria*

The total number of mesophilic aerobic bacteria in the sausage samples analyzed was varied widely; in most of them the count was above 6 $\log_{10}$ ufc.g$^{-1}$ (Table 1). Ambrosiadis et al.

**Table 1.** Results of the microbiological analysis of 50 sausage samples obtained by spontaneous fermentation and produced by different small manufacturers operating in the meat industry in the South of Brazil.

| | PCA | | MRS | | MSA | | DRBC | | BP | | VB | EC | EC-MUG |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | SD | M | SD | M | SD | M | SD | M | SD | | | |
| 1* | 8,48 | 0,08 | 6,65 | 0,06 | 6,58 | 0,06 | 5,95 | 0,02 | <1 | n.a | <3 | <3 | <3 |
| 2 | 8,36 | 0,07 | 6,64 | 0,08 | 7,57 | 0,03 | 6,20 | 0,09 | <1 | n.a | 93 | 93 | 43 |
| 3 | 8,04 | 0,06 | 5,73 | 0,01 | 7,46 | 0,02 | 6,81 | 0,01 | <1 | n.a | 23 | <3 | <3 |
| 4 | 7,52 | 0,11 | 6,49 | 0,07 | 7,40 | 0,02 | 4,56 | 0,04 | <1 | n.a | 43 | 7 | 7 |
| 5 | 8,40 | 0,01 | 6,77 | 0,10 | 6,61 | 0,08 | 6,08 | 0,01 | <1 | n.a | 9 | <3 | <3 |
| 6 | 5,95 | 0,01 | 4,24 | 0,34 | 6,61 | 0,08 | 3,68 | 0,09 | 4,15 | 0,20 | <3 | <3 | <3 |
| 7 | 5,00 | 0,01 | 5,00 | 0,01 | 5,70 | 0,04 | 2,00 | 0,01 | 3,72 | 0,17 | <3 | <3 | <3 |
| 8 | 7,76 | 0,01 | 6,95 | 0,04 | 7,72 | 0,02 | 6,54 | 0,08 | 6,13 | 0,16 | 4 | <3 | <3 |
| 9 | 8,14 | 0,08 | 7,52 | 0,06 | 7,40 | 0,04 | 6,51 | 0,04 | <1 | n.a | 9 | <3 | <3 |
| 10 | 8,11 | 0,02 | 7,70 | 0,04 | 7,83 | 0,02 | 5,75 | 0,12 | <1 | n.a | 9 | <3 | <3 |
| 11 | 7,23 | 0,08 | 7,34 | 0,01 | 5,76 | 0,05 | 3,78 | 0,08 | <1 | n.a | 23 | 4 | 4 |
| 12 | 7,85 | 0,07 | 7,76 | 0,07 | 6,16 | 0,01 | 4,20 | 0,02 | <1 | n.a | 23 | <3 | <3 |
| 13 | 7,76 | 0,13 | 7,64 | 0,05 | 7,33 | 0,02 | 4,12 | 0,03 | 5,48 | 0,01 | 23 | 4 | 4 |
| 14 | 8,33 | 0,02 | 6,97 | 0,03 | 7,59 | 0,04 | 5,71 | 0,06 | <1 | n.a | 240 | 23 | 23 |
| 15 | 7,12 | 0,03 | 6,87 | 0,01 | 6,33 | 0,05 | 2,39 | 0,12 | <1 | n.a | 23 | <3 | <3 |
| 16 | 6,89 | 0,02 | 5,99 | 0,02 | 6,81 | 0,09 | 3,27 | 0,27 | <1 | n.a | 9 | <3 | <3 |
| 17 | 7,16 | 0,01 | 6,06 | 0,03 | 7,22 | 0,04 | 4,71 | 0,12 | <1 | n.a | <3 | <3 | <3 |
| 18 | 6,13 | 0,12 | 4,24 | 0,34 | 6,35 | 0,10 | 4,19 | 0,06 | <1 | n.a | <3 | <3 | <3 |
| 19 | 6,16 | 0,06 | 5,96 | 0,10 | 5,81 | 0,01 | 4,24 | 0,09 | <1 | n.a | <3 | <3 | <3 |
| 20 | 8,22 | 0,01 | 8,02 | 0,06 | 5,00 | 0,08 | 3,66 | 0,26 | <1 | n.a | <3 | <3 | <3 |
| 21 | 8,04 | 0,09 | 7,87 | 0,03 | 5,09 | 0,03 | 5,92 | 0,07 | <1 | n.a | 9 | 9 | 9 |
| 22 | 8,01 | 0,01 | 7,73 | 0,07 | 7,40 | 0,07 | 5,56 | 0,12 | <1 | n.a | <3 | <3 | <3 |
| 23 | 7,66 | 0,01 | 7,47 | 0,01 | 5,90 | 0,02 | 5,25 | 0,02 | <1 | n.a | 23 | 4 | 4 |
| 24 | 8,07 | 0,12 | 7,60 | 0,17 | 5,67 | 0,01 | 4,89 | 0,01 | <1 | n.a | 4 | 4 | 4 |
| 25 | 6,74 | 0,07 | 5,84 | 0,05 | 5,48 | 0,12 | 5,86 | 0,09 | <1 | n.a | <3 | <3 | <3 |
| 26 | 7,58 | 0,08 | 4,00 | 0,01 | 7,22 | 0,03 | 5,42 | 0,05 | <1 | n.a | <3 | <3 | <3 |
| 27 | 7,65 | 0,03 | 7,46 | 0,02 | 6,33 | 0,04 | 5,21 | 0,06 | <1 | n.a | 23 | 23 | 23 |
| 28 | 8,41 | 0,02 | 7,98 | 0,09 | 7,05 | 0,04 | 4,94 | 0,07 | <1 | n.a | <3 | <3 | <3 |
| 29 | 8,34 | 0,17 | 7,91 | 0,05 | 7,23 | 0,03 | 5,43 | 0,18 | 5,72 | 0,17 | ≥2400 | ≥2400 | ≥2400 |
| 30 | 6,45 | 0,08 | 6,49 | 0,14 | 6,37 | 0,09 | 3,70 | 0,12 | 4,83 | 0,18 | 4 | 4 | 4 |
| 31 | 7,77 | 0,01 | 6,17 | 0,02 | 4,94 | 0,03 | 2,15 | 0,21 | <1 | n.a | <3 | <3 | <3 |
| 32 | 7,87 | 0,03 | 7,28 | 0,04 | 4,78 | 0,06 | 3,01 | 0,23 | <1 | n.a | <3 | 4 | 4 |
| 33 | 5,00 | 0,01 | 5,00 | 0,01 | 4,45 | 0,21 | 2,00 | 0,01 | <1 | n.a | <3 | <3 | <3 |
| 34 | 8,34 | 0,11 | 8,35 | 0,04 | 7,16 | 0,02 | 4,93 | 0,04 | 3,69 | 0,12 | 43 | 23 | 23 |
| 35 | 5,00 | 0,01 | 6,70 | 0,02 | 4,06 | 0,03 | 2,00 | 0,01 | <1 | n.a | 4 | <3 | <3 |
| 36 | 7,90 | 0,03 | 8,02 | 0,04 | 6,07 | 0,04 | 2,00 | 0,01 | 4,16 | 0,02 | 4 | <3 | <3 |
| 37 | 6,82 | 0,12 | 6,64 | 0,09 | 3,00 | 0,01 | 4,99 | 0,13 | <1 | n.a | <3 | <3 | <3 |
| 38 | 8,00 | 0,02 | 7,86 | 0,11 | 6,15 | 0,01 | 3,67 | 0,13 | 4,00 | 0,01 | 150 | 20 | 20 |
| 39 | 6,77 | 0,32 | 5,46 | 0,04 | 7,08 | 0,04 | 3,77 | 0,10 | <1 | n.a | 43 | <3 | <3 |
| 40 | 8,19 | 0,07 | 7,04 | 0,48 | 6,95 | 0,03 | 4,63 | 0,06 | <1 | n.a | 240 | 43 | 43 |
| 41 | 6,45 | 0,08 | 6,57 | 0,01 | 6,47 | 0,05 | 3,17 | 0,08 | 3,66 | 0,26 | <3 | <3 | <3 |
| 42 | 6,04 | 0,06 | 5,50 | 0,28 | 6,52 | 0,02 | 2,00 | 0,01 | 3,00 | 0,01 | <3 | <3 | <3 |
| 43 | 6,69 | 0,11 | 7,18 | 0,02 | 6,81 | 0,04 | 2,00 | 0,01 | 5,65 | 0,07 | <3 | <3 | <3 |
| 44 | 5,90 | 0,08 | 5,54 | 0,09 | 5,70 | 0,02 | 4,25 | 0,01 | 1,70 | 0,01 | 9 | <3 | <3 |
| 45 | 5,65 | 0,07 | 4,00 | 0,01 | 6,16 | 0,01 | 4,89 | 0,10 | <1 | n.a | 23 | <3 | <3 |
| 46 | 7,22 | 0,02 | 5,86 | 0,08 | 5,20 | 0,02 | 3,77 | 0,05 | <1 | n.a | ≥2400 | ≥2400 | ≥2400 |
| 47 | 7,52 | 0,06 | 7,25 | 0,02 | 7,29 | 0,04 | 2,00 | 0,01 | 4,70 | 0,01 | 240 | 240 | 240 |
| 48 | 7,67 | 0,01 | 7,74 | 0,01 | 7,15 | 0,04 | 3,97 | 0,05 | <1 | n.a | 4 | 4 | 4 |
| 49 | 5,00 | 0,01 | 4,00 | 0,01 | 4,00 | 0,01 | 2,00 | 0,01 | | | <3 | <3 | <3 |
| 50 | 6,96 | 0,02 | 6,34 | 0,06 | 6,96 | 0,08 | 4,26 | 0,07 | 4,26 | 0,01 | 93 | 93 | 93 |

*Sausages samples. The results of PCA (aerobic mesophilic cont), MRS (lactic acid bacteria), MSA (*Micrococcaceae*), DRBC (moulds and yeasts), and BP (coagulase-positive S*taphylococcus*) are expressed in $\log_{10}$ colony forming unit per gram ($CFU.g^{-1}$). The results of VB (total coliforms), EC (fecal coliforms), and EC-MUG (*E. coli*) are expressed in more probably number per gram ($MPN.g^{-1}$). M – mean of two measurements; SD – standard deviation; n.a – not applicable.

(2004), studying 67 samples of traditional sausages from Greece, found average values of 8 $\log_{10}$ ufc.g$^{-1}$ of mesophilic aerobic bacteria, with a minimum of 5 $\log_{10}$ and a maximum close to 9 $\log_{10}$ ufc.g$^{-1}$.

The mixture used in the production of sausages can have a very diverse microbiota, and it can also differ in terms of size of the initial population of the mixture. The initial differences are not always observed in the final product. In studies carried out to characterize typically Italian fermented sausages, the total count increased during maturation reaching values close to 7 $\log_{10}$ to 9 $\log_{10}$ ufc.g$^{-1}$ in the final product (MORETTI et al., 2004; COMI et al., 2005). The microorganisms present in the initial mixture come from the raw material, seasonings, utensils, and the environment (HOLZAPFEL, 2002; COMI et al., 2005).

The processing conditions and the addition of starter cultures result in final products with different microbial concentrations. In sausages, the total count of mesophilic aerobic bacteria was similar to the lactic bacteria counts, confirming the predominance of this group of microorganisms in the manufacture of these products (SANZ et al., 1997). On the other hand, in studies that evaluate the effects of the addition of starter cultures and additives on the quality of the sausage, the total aerobic mesophilic bacteria count increased during the first days of maturation and was around 8 $\log_{10}$ ufc.g$^{-1}$ at the end of maturation period. This treatment influenced the total quantity of aerobic bacteria (BOZKURT; ERKMEN, 2002).

In typical sausages from Turkey (sucuks) obtained by spontaneous fermentation, variations in the quantity of fat added to the formulations and the processing temperature changed significantly the total bacteria count, which was around 8 $\log_{10}$ ufc.g$^{-1}$ in the final product (SOYER; ERTAS; ÜZÜMCÜOGLU, 2005).

### *3.2 Lactic acid bacteria*

In the production of sausages, the rapid decrease in pH is important in order to avoid the development of deteriorative microorganisms and pathogens. The pH reduction depends on the rapid development of lactic bacteria. The mixture to produce sausages by spontaneous fermentation must contain a sufficient quantity of lactic bacteria and *Micrococcaceae*, which is necessary for the adequate development of the fermentation process, thus resulting in a safe and good quality product.

In sausages produced with the addition of starter cultures, the reduction in pH is more significant and stable, even when the quantity of lactic bacteria in the final product is similar to those produced by spontaneous fermentation. This is due to the higher acidification capacity of the starter cultures in comparison to that of native microbiota (SANZ et al., 1997).

The number of lactic bacteria at the beginning of fermentation varies greatly. These microorganisms multiply very fast and predominate during the first days of fermentation reaching values around 8 $\log_{10}$ ufc.g$^{-1}$. This count remains stable during maturation, with a possibility of decreasing in viability during the dehydration stage (SAMELIS et al., 1994; COPPOLA et al., 2000; PAPAMANOLI et al., 2002; MAURIELLO; CASABURI; VILLANI, 2004; GRECO et al., 2005; SOYER; ERTAS; ÜZÜMCÜOGLU, 2005; COMI et al., 2005).

In the sausage samples analyzed, the number of lactic bacteria varied considerably (Table 1). The samples with the smallest levels had counts lower than 4 $\log_{10}$ ufc.g$^{-1}$. However, in most samples (72%) the count of lactic bacteria was higher than 6 $\log_{10}$ ufc.g$^{-1}$, and the samples with the highest counts were above 8 $\log_{10}$ ufc.g$^{-1}$. The results obtained in the present study are similar to those found by Ambrosiadis et al. (2004), who found counts around 4 $\log_{10}$ to 8 $\log_{10}$ ufc.g$^{-1}$ in the characterization of traditional Greek sausages obtained by spontaneous fermentation.

The final number of lactic bacteria depends on the production conditions adopted, such as the binomial temperature and relative humidity. Moretti et al. (2004), studying the influence of these factors using two different maturation conditions, obtained different results, with counts ranging from 7 $\log_{10}$ to 8 $\log_{10}$ ufc.g$^{-1}$. In an experiment carried out by Sanz et al. (1997) to evaluate differences in time, temperature, and relative humidity during sausage maturation, the lactic bacteria count obtained in the final product was around 6 $\log_{10}$ ufc.g$^{-1}$, and during fermentation the maximal count was around 8 $\log_{10}$ ufc.g$^{-1}$.

In a study carried out by Soyer, Ertas and Üzümcüoglu (2005), the maturation temperature and the fat content affected the lactic bacteria levels, and the sausages produced with 10% fat presented the highest counts, followed by those with 20 and 30% fat.

The wide variation in the number of lactic bacteria among the samples of the evaluated sausages can be justified by the different production conditions adopted. In the Brazilian small sized meat product companies, during the maturation stage, these products are stored in non-controlled environmental conditions. Factors such as variation in the content of the main components in the formulations (fat and protein), the initial microbiota of the raw material, and of the added spices can also be the cause of these differences. Another important factor that might influence the number of lactic acid bacteria is the maturation time of fermented sausages. From the manufacturing date of the sausages analyzed in the present study, differences in the maturation time were observed. Some samples were commercialized soon after production, which resulted in short and inadequate maturation time, whereas other sausages at more than 21 days of maturation time showed higher lactic acid bacteria number.

The metabolic reactions caused by the growth of microorganisms in fermented sausages, mainly lactic acid bacteria, is the main responsible factor for the development of the final characteristics that are peculiar to these products. High numbers of lactic bacteria also contribute to the microbiological stability and safety of sausages by inhibiting the growth of pathogenic bacteria and deteriorative bacterial flora. The suppression of competitors by lactic bacteria occurs mainly due to changes in the substrate characteristics (pH and water activity) caused by the production of organic acids. Additionally,

some lactic acid bacteria produce certain substances such as bacteriocines, which are able to inhibit the growth of some microorganisms (HAMMES; BANTLEON; MIN, 1990; SAMELIS et al., 1994; HUGAS; MONFORT, 1997; HANSEN, 2002; HOLZAPFEL, 2002; TERRA, 2003; PAPAMANOLI et al., 2003; LEROY; VUYST, 2004).

### *3.3 Micrococcaceae*

The results of the number of *Micrococcaceae* in the fermented sausage samples analyzed are shown in Table 1. Like the total mesophyl aerobic bacteria and lactic acid bacteria counts, the *Micrococcaceae* count varied greatly among the studied samples. Most of the samples have counts ranging from 5 $\log_{10}$ to 7 $\log_{10}$ ufc.g$^{-1}$. Factors such as processing conditions, initial concentration of *Micrococcaceae* in the mixture, maturation time, number of lactic acid bacteria, and pH of the product can influence the final size of the *Micrococcaceae* population.

In general, the *Micrococcaceae* concentration reaches its maximum at the end of fermentation (SANZ et al., 1997). Data reported in the literature are controversial not only terms of the stage of maturation but also the *Micrococcaceae* final count in different kinds of sausage. In a study carried out by Papamanoli et al. (2002), the number of *Micrococcaceae* at the beginning of fermentation ranged from 3 $\log_{10}$ to 4 $\log_{10}$ ufc.g$^{-1}$, and this count did not change during maturation, remaining below 2 $\log_{10}$ ufc.g$^{-1}$ in the final product. In some studies on the characterization of spontaneously fermented sausages, the initial count of *Micrococcaceae* was 3-4 $\log_{10}$ ufc.g$^{-1}$ reaching around 6 $\log_{10}$ ufc.g$^{-1}$ in the final product (COPPOLA et al., 2000; MAURIELLO; CASABURI; VILLANI, 2004; COMI et al., 2005).

The maturation temperature affects significantly the final concentration of *Micrococcaceae*. During the period of fermentation, for sausages produced at temperatures between 24-26 °C, the *Micrococcaceae* count was around 7 $\log_{10}$ ufc.g$^{-1}$, whereas at 22-24 °C, the count was below 6 $\log_{10}$ ufc.g$^{-1}$. Under both conditions there was a decrease in the number of *Micrococcaceae* following the maturation period. The low number of *Micrococcaceae* during the production of fermented sausages, or the decrease during the maturation period may be due to the inhibition caused by a strong pH decrease, which was caused by the development of lactic acid bacteria (SOYER; ERTAS; ÜZÜMCÜOGLU, 2005). Sanz et al. (1997) found the highest count of *Micrococcaceae* in sausages with lower final count of lactic acid bacteria and, consequently, higher pH values.

In various studies on the characterization of fermented meat products, it was observed a predominance of *Staphylococcus* over other members of the *Micrococcaceae* family. The species of *S. saprophyticus*, *S. xylosus* e *S. carnosus* are predominant, and the last two are components of the starter cultures used in the preparation of fermented meat products. These species are used due to their metabolic reactions produced during their development, which contributes to the flavor and color formation in these products (MIRALLES; FLORES; PREZ-MARTINEZ, 1996; COPPOLA et al., 2000; GARCÍA-VARONA et al., 2000; PAPAMANOLI et al., 2002; MAURIELLO; CASABURI; VILLANI, 2004; CASABURI et al., 2005). The ability to produce antimicrobial compounds increases the interest in the use of these microorganisms in foods since they reduce deterioration and inhibit the growth of the pathogenic microorganisms, such as *Listeria monocytogenes* (PAPAMANOLI et al., 2002).

### *3.4 Molds and yeasts*

Some of the sausage samples analyzed presented mold and yeast counts below 2 $\log_{10}$ ufc.g$^{-1}$, and highest counts found were above 6 $\log_{10}$ ufc.g$^{-1}$ (Table 1). There was a predominance of yeast growth compared to that of the molds in all samples, and the latter developed mainly on the sausage surface as a natural consequence of the manufacturing technological process.

Various studies focused on the use of molds and yeasts or their enzymes in the production of sausages, the resulting metabolic reactions can contribute to the formation of aroma and to the final sensorial quality of sausages (ZALACAIN et al., 1995; 1996; SUNESEN; STAHNKE, 2003; ZAPELENA; ASTIASARÁN; BELLO, 1999; CASTRO; LUCHESE; MARTINS, 2000; BRUNA et al., 2003; FLORES et al., 2004; DURÁ; FLORES; TOLDRA, 2004).

The mold and yeast counts in the sausage samples analyzed are in accordance with those was found by Ambrosiadis et al. (2004). In a study carried out by Comi et al. (2005), the maximal count observed was around 3 $\log_{10}$ ufc.g$^{-1}$, and in some cases, the presence of molds and yeasts was not detected. Bozkurt and Erkmen (2002), in studies aiming at evaluating the effects of the addition of starter cultures and additives, observed that there was an increase in the number of molds and yeasts during the first days of maturation, reaching concentrations around 4 $\log_{10}$ ufc.g$^{-1}$ in treatments without the addition of additives. These numbers decreased during the further stages of maturation and storage. Other studies found molds and yeasts counts around 6 $\log_{10}$ ufc.g$^{-1}$ in the final product (SAMELIS et al., 1994; COPPOLA et al., 2000).

### *3.5 Total coliforms, fecal coliforms and E. coli*

High quality meat is of great importance in the production of sausages in order to obtain a final product with low counts of pathogenic bacteria, which reflects its sanitary conditions. Usually the number of *Enterobacteriaceae* decreases with maturation (SAMELIS et al., 1998; MORETTI et al., 2004), but in some cases, there is an increase in their number (COMI et al., 2005).

As for the fecal coliforms, 31 samples had <3 NMP.g$^{-1}$ values, 17 samples had values ranging from 3 to 240 NMP.g$^{-1}$, and 2 samples had values above 2.400 NMP.g$^{-1}$ (Table 1). The Brazilian legislation establishes a maximum of 3 $\log_{10}$ NMP.g$^{-1}$ for fecal coliforms (BRASIL, 2001), and thus, two samples were considered unsafe for consumption. High counts of these microorganisms indicate inadequate processing and/or recontamination after processing, and the most frequent causes are contamination by raw materials, dirty equipment, or poor hygiene in handling practices (TILDEN JUNIOR et al., 1996).

The presence of *E. coli* was confirmed in 38% of the samples. Higher values have been found in other studies, in which it was

found that 84% of the samples were contaminated with *E. coli* (MAGNANI et al., 2000; VIOTT; STOLBERG; PELISSER, 2006). The presence of *E. coli* in foods must be evaluated considering two aspects. First of all, it is an *Enterobacteriaceae*, which means that this food has a fecal origin contamination, and the other aspect to be considered is that various strains of *E. coli* have been proven pathogenic to humans (INCZE, 1998; FRANCO; LANDGRAF, 2003; MOORE, 2004).

### *3.6 Coagulase-positive Staphylococcus*

The results of the coagulase-positive *Staphylococcus* quantification in the 50 sausage samples analyzed are shown in Table 1. The presence of coagulase-positive *Staphylococcus* was confirmed in 15 samples (30%), and 11 had counts above the maximum allowed by the current legislation (3.7 $\log_{10}$ ufc.g$^{-1}$), thus reflecting unsafe sanitary conditions for consumption (BRASIL, 2001). Even in samples with quantities lower than the allowed limit, it does not mean that there is total absence of enterotoxins since initially, they could be present in sufficient number to produce toxins, and their population decreased during the fermentation process (MOORE, 2004). In another study, the presence of coagulase-positive *Staphylococcus* in sausages was observed in counts higher or equal to 6 $\log_{10}$ UFC.g$^{-1}$ in 45% of the samples analyzed (LOBO et al., 2001).

### *3.7 Salmonella spp.*

All sausage samples analyzed in the present study showed an absence of *Salmonella* spp. in 25 grams, complying with the current legislation (BRASIL, 2001). In studies carried out by Lobo et al. (2001), and Hoffman et al. (1997), aiming at evaluating the quality of sausages, the presence of *Salmonella* spp. was observed in 5% and 13.3% of the analyzed samples, respectively.

## 4 Conclusions

From the data obtained in the present study, it can be concluded that 24% of the samples analyzed do not complying with the legal requirements in Brazil since the counts of fecal coliforms or coagulase-positive *Staphylococcus* are above the maximum limit established. These results indicates the need for the implementation of programs aiming at sanitary condition improvement in small sausage manufacturing industries, as well as a more efficient government inspection focusing on eliminating potential health risks to the consumers.

The sausages produced by spontaneous fermentation and manufactured by small meat product producers in Brazil have a technologically important population of microorganisms (lactic bacteria, *Micrococcaceae*, molds, and yeasts) similar to that of other types of fermented sausages already described in the literature.

The use of controlled environmental conditions of production, as well as the standardization of the maturation time can contribute to the production of sausages with less characteristic variation and better final sanitary quality, reducing the possibility of occurring foodborne disease outbreaks associated to these products.

## References

AMBROSIADIS, J. et al. Physicochemical, microbiological and sensory attributes for the characterization of Greek traditional sausages. **Meat Science**, v. 66, p. 279-287, 2004. http://dx.doi.org/10.1016/S0309-1740(03)00100-1

BOZKURT, H.; ERKMEN, O. Effects of starter cultures and additives on the quality of Turkish style sausage (sucuk). **Meat Science**, v. 61, p. 149-156, 2002. http://dx.doi.org/10.1016/S0309-1740(01)00176-0

BRASIL. Ministério da Agricultura e do Abastecimento. Instrução Normativa nº 22 de 31 de junho de 2000. Aprovar os Regulamentos Técnicos de Identidade e Qualidade de Copa, de Jerked Beef, de Presunto tipo Parma, de Presunto Cru, de Salame, de Salaminho, de Salame tipo Alemão, de Salame tipo Calabres, de Salame tipo Friolano, de Salame tipo Napolitano, de Salame tipo Hamburgues, de Salame tipo Italiano, de Salame tipo Milano, de Linguiça Colonial e Pepperoni. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 03 ago. 2000a. Anexo V - Regulamento Técnico de Identidade e Qualidade de Salame. Disponível em: <http://www.engetecno.com.br/legislacao/carnes_salame.htm>. Acesso em: 24 jun. 2006.

BRASIL. Ministério da Agricultura e do Abastecimento. Instrução Normativa nº 22 de 31 de junho de 2000. Aprovar os Regulamentos Técnicos de Identidade e Qualidade de Copa, de Jerked Beef, de Presunto tipo Parma, de Presunto Cru, de Salame, de Salaminho, de Salame tipo Alemão, de Salame tipo Calabres, de Salame tipo Friolano, de Salame tipo Napolitano, de Salame tipo Hamburgues, de Salame tipo Italiano, de Salame tipo Milano, de Linguiça Colonial e Pepperoni. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 03 ago. 2000b. Anexo XIV - Regulamento Técnico de Identidade e Qualidade da Linguiça Colonial. Disponível em: <http://www.engetecno.com.br/legislacao/carnes_salame.htm>. Acesso em: 24 jun. 2006.

BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária – ANVISA. Resolução nº 12, de 2 de janeiro de 2001. Regulamento técnico sobre padrões microbiológicos para alimentos. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 10 jan. 2001. Disponível em: <http://www.anvisa.gov.br/legis/resol/12-01.rdc.htm>. Acesso em: 24 jun. 2006.

BRUNA, J. M. et al. Changes in selected biochemical and sensory parameters as affected by the superficial inoculation of *Penicillium camemberti* on dry fermented sausages. **International Journal of Food Microbiology**, v. 85, p. 111-125, 2003. http://dx.doi.org/10.1016/S0168-1605(02)00505-6

BUCKENHÜSKES, H. J. Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. **FEMS Microbiology Reviews**, v. 12, p. 253-272, 1993. http://dx.doi.org/10.1016/0168-6445(93)90067-J

CASABURI, A. et al. Technological activities of *Staphylococcus carnosus* and *Staphylococcus simulans* strains isolated from fermented sausages. **Meat Science**, v. 71, p. 643-650, 2005. PMid:22061209. http://dx.doi.org/10.1016/j.meatsci.2005.05.008

CASTRO, L. C.; LUCHESE, R. H.; MARTINS, J. F. P. Efeito do uso da cepa starter de *Penicillium nalgiovense* na qualidade de salames. **Ciência e Tecnologia de Alimentos**, v. 20, p. 40-46, 2000. http://dx.doi.org/10.1590/S0101-20612000000100009

COMI, G. et al. Characterization of naturally fermented sausage produced in the North East of Italy. **Meat Science**, v. 69,

p. 381-392, 2005. PMid:22062975. http://dx.doi.org/10.1016/j.meatsci.2004.08.007

COPPOLA, S. et al. Microbial succession during ripening of Naples-type salami, a southern Italian fermented sausage. **Meat Science**, v. 56, p. 321-329, 2000. http://dx.doi.org/10.1016/S0309-1740(00)00046-2

DURÁ, M. A.; FLORES, M.; TOLDRÁ, F. Effect of *Debaryomyces* spp. on the proteolysis of dry-fermented sausages. **Meat Science**, v. 68, p. 319-328, 2004. PMid:22062243. http://dx.doi.org/10.1016/j.meatsci.2004.03.015

FLORES, M. et al. Effect of *Debaryomyces* spp. on aroma formation and sensory quality of dry-fermented sausages. **Meat Science**, v. 68, p. 439-446, 2004. PMid:22062412. http://dx.doi.org/10.1016/j.meatsci.2003.04.001

FRANCO, B. D. G. M.; LANDGRAF, M. **Microbiologia dos Alimentos**. São Paulo: Atheneu, 2003.

FRANCO, I. et al. Study of the biochemical changes during the processing of Androlla, a Spanish dry-cured pork sausage. **Food chemistry**, v. 78, p. 339-345, 2002. http://dx.doi.org/10.1016/S0308-8146(02)00118-8

GARCÍA-VARONA, M. et al. Characterization of *Micrococcaceae* isolated from different varieties of chorizo. **International Journal of Food Microbiology**, v. 54, p. 189-195, 2000. http://dx.doi.org/10.1016/S0168-1605(99)00192-0

GRECO, M. et al. Evolution and identification of lactic acid bacteria isolated during the ripening of Sardinian sausages. **Meat Science**, v. 69, p. 733-739, 2005. PMid:22063151. http://dx.doi.org/10.1016/j.meatsci.2004.11.004

HAMMES, W. P.; BANTLEON, A.; MIN, S. Lactic acid bacteria in meat fermentation. **FEMS Microbiology Reviews**, v. 87, p. 165-174, 1990. http://dx.doi.org/10.1111/j.1574-6968.1990.tb04886.x

HANSEN, E. B. Commercial bacterial starter cultures for fermented foods of the future. **International Journal of Food Microbiology**, v. 78, p. 119-131, 2002. http://dx.doi.org/10.1016/S0168-1605(02)00238-6

HOFFMANN, F. L. et al. Qualidade microbiológica de amostras de salame. **Boletim do CEPPA**, v. 15, p. 57-64, 1997.

HOLZAPFEL, W. H. Appropriate starter culture technologies for small-scale fermentation in developing countries. **International Journal of Food Microbiology**, v. 75, p. 197-212, 2002. http://dx.doi.org/10.1016/S0168-1605(01)00707-3

HUGAS, M.; MONFORT, J. M. Bacterial starter cultures for meat fermentation. **Food Chemistry**, v. 59, p. 547-554, 1997. http://dx.doi.org/10.1016/S0308-8146(97)00005-8

INCZE, K. Dry Fermented Sausages. **Meat Science**, v. 49, p. 169-177, 1998. http://dx.doi.org/10.1016/S0309-1740(98)90046-8

LEROY, F.; VUYST, L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. **Trends in Food Science & Technology**, v. 15, p. 67-78, 2004. http://dx.doi.org/10.1016/j.tifs.2003.09.004

LOBO, M. V. et al. Avaliação microbiológica de salames coloniais comercializados no município de Santa Maria – RS. **Higiene Alimentar**, v. 88, p. 57-61, 2001.

LÜCKE, F-K. Utilization of microbes to process and preserve meat. **Meat Science**, v. 56, p. 105-115, 2000. http://dx.doi.org/10.1016/S0309-1740(00)00029-2

MAGNANI, A. L. et al. Incidência de *Salmonella* e *Escherichia coli* em carne suína in natura e salame colonial, consumidos pela população de Chapecó-SC. **Revista Higiene Alimentar**, v. 73, p. 44-47, 2000.

MAURIELLO, G.; CASABURI, A.; VILLANI, F. Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy. **Meat Science**, v. 67, p. 149-158, 2004. PMid:22061128. http://dx.doi.org/10.1016/j.meatsci.2003.10.003

MIRALLES, M. C.; FLORES, J.; PREZ-MARTINEZ, G. Biochemical tests for the selection of *Staphylococcus* strains as potential meat starter cultures. **Food Microbiology**, v. 13, p. 227-236, 1996. http://dx.doi.org/10.1006/fmic.1996.0028

MOORE, J. E. Gastrointestinal outbreaks associated with fermented meats. **Meat Science**, v. 67, p. 565-568, 2004. PMid:22061805. http://dx.doi.org/10.1016/j.meatsci.2003.12.009

MORETTI, V. M. et al. Chemical and microbiological parameters and sensory attributes of a typical Sicilian salami ripened in different conditions. **Meat Science**, v. 66, p. 845-854, 2004. PMid:22061017. http://dx.doi.org/10.1016/j.meatsci.2003.08.006

NASSU, R. T.; GONSALVES, L. A. G.; BESERRA, F. E. Efeito do teor de gordura nas características químicas e sensoriais de embutido fermentado de carne de caprinos. **Pesquisa Agropecuária Brasileira**, v. 32, p. 1169-1173, 2002. http://dx.doi.org/10.1590/S0100-204X2002000800015

OLIVEIRA, K. A. M.; MENDONÇA, R. C. S. Efeito da fermentação sobre a microbiota de embutidos cárneos. **Revista Higiene Alimentar**, v. 123, p. 12-17, 2004.

PAPAMANOLI, E. et al. Characterization of *Micrococcaceae* isolated from dry-fermented sausage. **Food Microbiology**, v. 19, p. 441-449, 2002. http://dx.doi.org/10.1006/fmic.2002.0503

PAPAMANOLI, E. et al. Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. **Meat Science**, v. 65, p. 859-867, 2003. http://dx.doi.org/10.1016/S0309-1740(02)00292-9

PEREIRA, K. S. Patógenos bacterianos em salames. **Revista Nacional da Carne**, v. 328, p. 23-24, 2004.

SAMELIS, J. et al. Stability and safety of traditional Greek salami – a microbiological ecology study. **International Journal of Food Microbiology**, v. 44, p. 69-82, 1998. http://dx.doi.org/10.1016/S0168-1605(98)00124-X

SAMELIS, J. et al. Quantification and characterization of microbial populations associated with naturally fermented Greek dry salami. **Food Microbiology**, v. 11, p. 447-460, 1994. http://dx.doi.org/10.1006/fmic.1994.1050

SANTOS, E. M. et al. Comparative study of lactic acid bacteria house flora isolated in different varieties of 'chorizo'. **International Journal of Food Microbiology**, v. 39, p. 123-128, 1998. http://dx.doi.org/10.1016/S0168-1605(97)00128-1

SANZ, Y. et al. Effect of pre-ripening on microbial and chemical changes in dry fermented sausages. **Food Microbiology**, v. 14, p. 575-582, 1997. http://dx.doi.org/10.1006/fmic.1997.0121

SOYER, A.; ERTAS, A. H.; ÜZÜMCÜOGLU, Ü. Effects of processing conditions on the quality of naturally fermented Turkish sausages (Sucuks). **Meat Science**, v. 69, p. 135-141, 2005. PMid:22062649. http://dx.doi.org/10.1016/j.meatsci.2004.06.015

SUNESEN, L. O.; STAHNKE, L. H. Mould starter cultures for dry sausages-selection, application and effects. **Meat Science**, v. 65, p. 935-948, 2003. http://dx.doi.org/10.1016/S0309-1740(02)00281-4

TERRA, N. N. **Apontamentos de Tecnologia de Carnes**. São Leopoldo: Unisinos, 1998.

TERRA, N. N. Particularidades na fabricação de salame. **Revista Nacional da Carne**, v. 317, p. 12-22, 2003.

TILDEN JUNIOR, J. et al. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. **American Journal of Public Health**, v. 86, p. 1142-1145, 1996. http://dx.doi.org/10.2105/AJPH.86.8_Pt_1.1142

TYÖPPÖNEN, S. et al. Survival of *Listeria Monocytogenes* in North European type dry sausages fermented by bioprotective meat starter cultures. **Food Control**, v. 14, p. 181-185, 2003. http://dx.doi.org/10.1016/S0956-7135(02)00086-5

VIOTT, A.; STOLBERG, J.; PELISSER, M. R. Qualidade microbiológica e físico-química de salames tipo coloniais da região do alto Uruguai catarinense. **Higiene Alimentar**, v. 138, p. 78-83, 2006.

ZALACAIN, I. et al. Dry fermented sausage elaborated with lipase from *Candida cylindracea*. Comparison with traditional formulations. **Meat Science**, v. 40, p. 55-61, 1995. http://dx.doi.org/10.1016/0309-1740(94)00023-Z

ZALACAIN, I. et al. Addition of lipase from *Candida cylindracea* to a traditional formulation of a dry fermented sausage. **Meat Science**, v. 42, p. 155-163, 1996. http://dx.doi.org/10.1016/0309-1740(95)00033-X

ZAPELENA, M. J.; ASTIASARÁN, I.; BELLO, J. Dry fermented sausages made with a protease from *Aspergillus oryzae* and/or a starter culture. **Meat Science**, v. 52, p. 403-409, 1999. http://dx.doi.org/10.1016/S0309-1740(99)00022-4