LACTOBACILLUS ACIDOPHILUS AND *BIFIDOBACTERIUM LACTIS* MICROENCAPSULATED IN DRY FERMENTED SAUSAGE

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Abstract – Probiotics has become widely accepted as a natural approach to promote human health. *Lactobacillus* and bifidobacteria are considered to be the best microorganisms for use as probiotics in meat products. In order to study this effect in a food system, microencapsulated probiotics by spray chilling was added in salami. Its effect was verified along the processing on some meat product quality characteristics, such as pH, lactic acidity, lipid oxidation, water activity, and weight loss. These results were compared to the control sample with no probiotics added. The results showed that microencapsulated probiotics did not affect the quality characteristics and it was efficient in controlling the lactic acidity in the final product.

Key Words – probiotics, salami, spray chilling

I. INTRODUCTION

In the last decade, health has become a major concern among scientists worldwide. Due to this fact, there is a trend of using probiotic foods in the food industry. [1]. The use of probiotics seems to be more promising in raw fermented products because it can be consumed without prior heating, which would lead the probiotics to death [2]. Dry fermented sausages are the result of several factors that act in synergy with biochemical, microbiological, physical, and sensorial characteristics during ripening. Also, it is important to mention that drying occurs during the ripening of the product. This process favors the growth of microorganisms which influences the safety and the sensorial and nutritional qualities. [3]. Microorganisms are sensitive to a variety of factors, such as acid, the presence of oxygen and antimicrobial compounds. Microencapsulation has proven to be a viable alternative to ensure the viability of probiotics in dry fermented sausages. In addition, it may promote their controlled release in the intestine. Moreover, it prevents these microorganisms to be multiplied in food, changing its sensory characteristics [4]. Hence, microencapsulated probiotics add more value to salami, if it is incorporated. The aim of this study was to define the effects of using L. *acidophilus* and *B. lactis* microencapsulated on the quality characteristics of salami.

II. MATERIALS AND METHODS

The Italian salami was prepared using the following composition: pork shoulder (60%), beef rib (20%) and pork backfat (20%), sodium nitrite (0.015%) and nitrate (0.015%), sodium chloride (2.5%), sugar (0.4%), dextrose (0.75%), maltodextrin (0.5%), sodium erythorbate (0.5%), monosodium glutamate (0.1%), white pepper (0.2%), nutmeg (0.1%), coriander (0.1%), garlic powder (0.2%) and starter culture (0.0125%) Bactoferm T-SPX containing *Staphylococcus xylosus* and *Pediococcus pentosaceus* (Chr. Hansen ®). The probiotics microcapsules were prepared by the College of Animal Science and Food Engineering from University of Sao Paulo (USP). In this project, the microencapsulated probiotics were prepared with (1%) *Lactobacillus*

acidophilus and *Bifidobacterium lactis* (Danisco ®) by spray chilling [5]. Meat was ground and mixed with non-meat ingredients to obtain a homogeneous mass, which was embedded in reconstituted collagen casing. One batch of 40 kg was prepared (8kg per treatment): T1 (control without probiotics), T2 (with 1% of *L. acidophilus* free), T3 (with 1% of *L. acidophilus* microencapsulated), T4 (with 1% of *B. lactis* free) and T5 (with 1% of *B. lactis* microencapsulated). The pieces were fermented in a climatic chamber until pH between 5.0 and 5.2 and then dried and ripening for up to 13 days. Oxidation lipid (TBARS values) of salami, expressed in mg malonaldehyde/kg sample, was determined during the processing [6]. Water activity was determined by AquaLab 4T analyzer (Decagen Devices, USA) at 25°C ± 0.3. pH values were determined using a pH meter (pH 300, USA) with automatic temperature compensation and a glass penetration electrode. Lactic acid was determined on the neutralization of free hydrogen ions, to the point of equivalence, by sodium hydroxide in the presence of phenolphthalein indicator [7]. Weight loss was calculated daily (13 days) with a semi-analytical balance (Marte AL500C, Brazil), through randomly selected of 3 pieces of salami each treatment. The obtained data was statistically analyzed by analysis of variance ANOVA ($p \leq 0.05$) using the Tukey HSD test ($p \leq 0.05$). The statistical data analysis was performed using STATISTICA™ software (Statsoft Inc., U.S.A.).

III. RESULTS AND DISCUSSION

Considering the results in mass variance analysis no significant difference ($p>0.05$) among the average of five treatments was found. The average from 6.2 to 6.3 (Table 1), was similar to the range of 6.35 to 6.65 as it was observed in Italian salamis. This similarity may be due to raw material and formulation employed [8]. In salami ripening and dry treatment T2 (*L. acidophilus* free) showed an average of 4.8. The over-fermentation of L. *acidophilus* is due to the fact of generating compounds that stimulate the activity of other lactic acid bacteria. The average of 5.2 for T5 (*B. lactis* microencapsulated) is possibly related to the reduction of their metabolic activity which is responsible for the process. Since they were encapsulated, they had no access to nutrients that could promote its fermentation, maintaining the pH above 5.0.

Table 1. pH (± standard deviations) in mass samples, salami day 0 (matured and dry) and 30 days of processing

Treat-ments	pH		
	Mass	0 (salami)	30
T1	$6.2^{a} \pm 0.24$	$5.3^{a} \pm 0.10$	$5.4^{a} \pm 0.08$
T2	$6.2^{a} \pm 0.25$	$4.8^{b} \pm 0.21$	$4.8^{b} \pm 0.30$
T3	$6.3^{a} \pm 0.17$	$5.2^{a} \pm 0.06$	$5.2^{ab} \pm 0.25$
T4	$6.3^{a} \pm 0.06$	$4.8^{b} \pm 0.07$	$4.8^{b} \pm 0.17$
T5	$6.3^{a} \pm 0.22$	$5.2^{a} \pm 0.08$	$5.2^{ab} \pm 0.22$

Averages followed by different letters in the same column differ significantly ($p \leq 0.05$) by the Tukey HSD test.

T1 (control), T3, and T5 (both microencapsulated) differed significantly in terms of pH values ($p \leq 0.05$) from other treatments. These results are compatible to the value of 5.3 to *L. reuteri* microencapsulated for the same period [9]. At 30 days of processing, a significant statistical difference was observed among treatments ($p \leq 0.05$). The highest pH averages were found for T1, T3 and T5 (both microencapsulated) (Table 1). The results as described above were found for *L. reuteri* microencapsulated with mean 4.7 [9].

This difference may have happened because the capsule could have released the probiotics into the salami. It is possible that this event did not occur in our study.

Likewise, the acidity of salami (day 0) in T2 and T4 (both free) (Figure 1) differed significantly ($p<0.05$) from other treatments and showed average values of 0.26. The evolution of titratable acidity occurred inversely proportional to the development of pH throughout the study period, which favors the confirmation of these results. Thus, it indicates consistency among the analyzed parameters. This fact can be attributed to lactic acid production by lactic acid bacteria

and indicate greater activity on the fermentation sugars (sucrose and glucose).

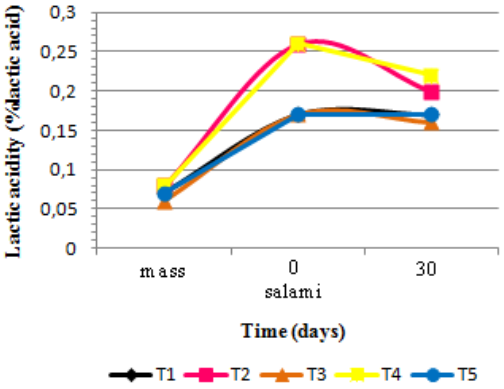

Figure 1. Lactic acidity values (% lactic acid) from samples for 30 days of salami processing

Figure 2 shows the results of TBARS during processing. On day 0 of shelf life, the salami presented a mass reduction in lipid oxidation, indicating that the monosodium erythorbate, which was added to the formulation, exerted an antioxidant effect on lipid rancidity. Therefore, there was no significant difference between treatments ($p>0.05$). The T2 and T4 (both free) showed the highest mean reaching concentrations of 0.6 mg of MDA/kg and 0.8 mg of MDA/kg, respectively. These values were higher than the values found in 0.33 mg of MDA/kg, for the same period [10].

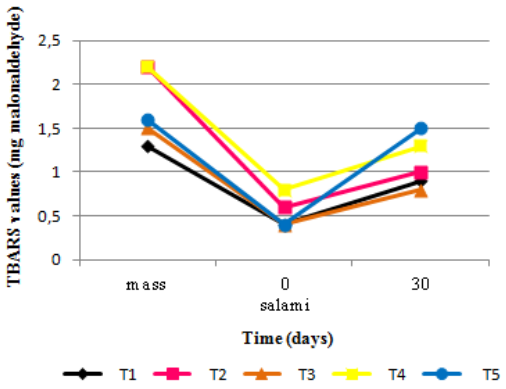

Figure 2. TBARS values (mg malonaldehyde) from samples for 30 days of salami processing

On day 30 of shelf life, the treatments T4 and T5 presented the highest levels of TBARS and differed significantly ($p<0.05$) from T1 and T3. In a 30 day period, there was a significant difference between treatments ($p<0.05$). T4 and the T5 demonstrated the highest average statistically different from T1 and T3. The increase of TBARS during storage is probably due to the action of lipolytic enzymes that release free unsaturated fatty acids, especially linoleic , oleic and arachidonic acids. These acids are highly susceptible to oxidation in meat products and are influenced by several factors related to the process of manufacturing, the amount and type of fat used (the microcapsules were coated interesterified palm oil), the content of salt and spices, the degree of grinding of the meat, the ripening temperature, and the pH and redox potential during the processing.

Water activity values (table 2) in salami (day 0) showed a statistical significant difference between treatments. Samples of T5 *lactis* encapsulated) differred significantly ($p<0.05$) from T2 and T4 (both free). The results of treatments are similar to the values of 0.88 for samples of salami with *L. reuteri* microencapsulated [9].

Table 2. Water activity values (± standard deviations) in mass samples, salami day 0 (matured and dry) and 30 days of processing

Treat-ments	Water activity		
	Mass	0 (salami)	30
T1	$0.97^{a} \pm 0.00$	$0.87^{ab} \pm 0.01$	$0.86^{a} \pm 0.00$
T2	$0.97^{a} \pm 0.00$	$0.84^{bc} \pm 0.00$	$0.84^{ab} \pm 0.01$
T3	$0.97^{a} \pm 0.00$	$0.86^{abc} \pm 0.00$	$0.86^{a} \pm 0.00$
T4	$0.96^{a} \pm 0.00$	$0.83^{c} \pm 0.01$	$0.83^{b} \pm 0.00$
T5	$0.96^{a} \pm 0.00$	$0.88^{a} \pm 0.01$	$0.86^{a} \pm 0.00$

Averages followed by different letters in the same column differ significantly ($p \leq 0.05$) by the Tukey HSD test

The highest values were found in mean from time 30 for T1 (control), T3 and T5 (both microencapsulated), differing significantly from T2 and T4 ($p<0.05$). These parameters are compatible to those found in the determination of pH, where these treatments showed the highest values. The pH is determined primarily by lactate, which reduces pH and increases ammonia. The water content interacts with proteins, resulting in a buffer effect. This event

explains the water activity values and pH, indicating consistency between the parameters analyzed.

In the analysis of data variance weight loss, significant effects of treatment ($p \leq 0.05$) and ripening time ($p \leq 0.05$) were observed. Therefore, there was no interaction effect of treatment versus aging time ($p > 0.05$). Figure 3 shows weight loss of sausages at 13 days of fermentation and drying. In this figure, we clearly observe the weight loss followed by exponential curves, where T2 and T4 (both free) presented a higher rate of weight loss (36.9% for *L. acidophilus* and 35.9% for *B. lactis*) during drying than T3 and T5 (both microencapsulated) 33.5% for *L. acidophilus* and 33.9% for B. Lactis, Both probiotic cultures *L. acidophilus* (T3) and *B. lactis* (T5) microencapsulated showed a similar weight loss to T1 (control).

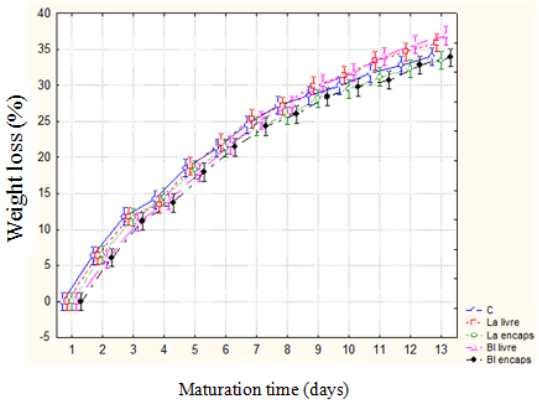

Figure 3. Weight loss (%) of samples of Italian salami during the 13 days drying

IV. CONCLUSION

Probiotic microencapsulation showed positive and relevant results whereby it may become a prominence in fermented sausages. The microencapsulated treatments did not reveal high acidity values as well as free, which could affect the sensory quality by consumers. In regard of weight loss, these same microencapsulated treatments showed similar losses to the control, corroborating with a positive result.

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