## PE8.02 Addition of microencapsulated propolis to dry fermented sausage 8.00

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Abstract-additives considered natural are increasingly being studied and used in foods, especially in meat and meat products. Propolis has various biological properties such as antioxidant. To study this effect in a food system, microencapsulated propolis through spray-drying technique and with two distinct encapsulating materials (OSA modified starch and arabic gum) was added in salami, evaluating the effect along the process on some quality product characteristics such as pH, lactic acidity, color change (L\*-brightness, a\*-red intensity) and lipid oxidation, with the determination of Thiobarbituric Acid Reactive Substances (TBARS), comparing with the results of a control sample, which was made without the addition of antioxidants. The results have proved to be satisfactory, as the propolis had not adversely affected the quality characteristics and was efficient in controlling the lipid oxidation in the final product.

*Index Terms*—fermented sausage, lipid oxidation, propolis, quality.

## I. INTRODUCTION

**URRENTTLY**, there has been a great interest by consumer market in purchasing products considered increasingly healthy or natural. Thus, with the aim of increasing the quality of meat products, an increasing interest in identifying and using natural antioxidants, replacing synthetic products has been observed [1]. Propolis is a natural resinous substance collected by bees from branches, flowers, pollen, buds and exudates of trees [2] and several studies have been published reviewing and disseminating the biological properties of propolis as antimicrobial, antifungal, antiprotozoan, antioxidant and antiviral [3], and flavonoid and phenolic compounds are responsible for its high antioxidant activity [4]. Among the obstacles to its use in food are the strong flavor and the difficulty of solubilization, which normally requires its marketing in alcoholic solutions forms. However, microencapsulation through spray-drying technique can solve such limitations due to its ability to overcome undesirable flavors, reduce volatility and reactivity and increase its stability under adverse environmental conditions [5]. The prospect for using propolis as a natural antioxidant in foods seems to be promising due to its antimicrobial and antioxidant properties. In this context, microencapsulated propolis incorporated in salami, which is a raw, fermented and dry sausage, may add more value to this product. Based on these results, the effects of its implementation on the quality characteristics of salami were studied.

## II. MATERIALS AND METHODS

### A. Formula

The mixture for salami was prepared using the following formula (%w/w): pork shoulder (57.64), pork backfat (19.21), beef chuck (19.21), sodium nitrate (0.0144) and nitrite (0.0096), salt (2.4), dextrose (0.72), sodium tripolyphosphate (0.29), monosodium glutamate (0.096), white pepper (0.19), coriander (0.096), garlic (0.096) and starter culture (0.012) of *Staphylococcus xylosus* and *Pediococcus pentosaceus* (Chr. Hansen®).

## B. Active Material

Microencapsulated through spray-drying technique, type-12 propolis was added, setting up 7 treatments according to the encapsulating material and concentration of microcapsules, which were: 1000 (T1), 1500 (T2) and 2000 ppm (T3) of propolis:OSA starch (1:6) microcapsules, 1500 (T4), 2500 (T5) and 4000 ppm (T6) of propolis:arabic gum (1:6) microcapsules and a control with 0% (T7). The propolis microcapsules were prepared and provided by Faculdade de Zootecnia e Engenharia de Alimentos (FZEA) -University of São Paulo (USP) in a partnership project, being prepared with a share of propolis extract with 19% of solids for six shares of 30% solution (w/w) of encapsulating material. The concentration of microcapsules added was calculated based on the antioxidant activity of the material, determined on a parallel project.

## C. Production of fermented sausages

Meat was ground and mixed with nonmeat ingredients to obtain a homogeneous mass, which was embedded in reconstituted collagen casing. The pieces were fermented in a climatic chamber until pH reached between 5.0 and 5.2 and then dried and matured for up to 30 days.

### D. Analysis

The lipid oxidation (TBARS values) of salami, expressed in mg malonaldehyde(MDA)/kg sample, was determined during the processing in accordance with methodology described by Hoz, D'Árrigo, Cambero and Ordóñez [6]. The water activity was determined by Aqua Lab 3 analyzer (Decagen Devices, USA) at 25°C ± 0.3. The L\* (brightness) and a\* (red intensity) parameters from the CIELAB system were obtained using portable colorimeter Minolta Chroma (Cr-400 model). To determine the lactic acidity, the methodology proposed by the Ministério da Agricultura e do Abastecimento [7] was used. The data obtained were statistically analyzed through analysis of variance ANOVA and Tukey test, using statistical program SAS (Statistic Analisy System), with 95% of confidence (p<0.05). It was used 3 samples of each treatment.

# III. RESULTS AND DISCUSSION

Salamis were dried until water activity of 0.92 and final medium pH around 5.1, as can be observed in Table 1, which also shows the lactic acidity results. It is observed that at the end of fermentation, the pieces had average pH around 5.0, resulting from lactic acid formation by lactic acid bacteria, from dextrose. According to Demeyer, Vandekerckhove & Moermans [8], pH is primarily determined by lactate, which lowers pH, ammonia, which increases and water content in interaction with proteins, in buffer effect. This explains the variations observed at the end of the fermentation phase in the final product.

**Table 1.** % lactic acid (%LA) and pH in stuffed mass, end of the fermentation and salami sample at the end of 30 days of processing.

<u> </u>	Mass		End of fermentation		Salami	
_	%LA	pН	%LA *	pН	%LA	pН
T1	0.22 <sup>b</sup>	6.40 <sup>b</sup>	-	5.05 <sup>bc</sup>	1.48 <sup>b</sup>	5.06 <sup>d</sup>
T2	$0.25^{ab}$	$6.47^{a}$	-	4.90 <sup>c</sup>	1.54 <sup>ab</sup>	4.88 <sup>e</sup>
Т3	$0.26^{ab}$	6.19 <sup>e</sup>	-	4.88 <sup>c</sup>	1.52 <sup>b</sup>	4.83 <sup>f</sup>
T4	0.28 <sup>a</sup>	6.37 <sup>d</sup>	-	5.13 <sup>ab</sup>	1.49 <sup>b</sup>	5.24 <sup>a</sup>
T5	$0.26^{ab}$	6.48 <sup>a</sup>	-	5.13 <sup>ab</sup>	1.62 <sup>a</sup>	5.24 <sup>a</sup>
T6	$0.27^{ab}$	6.38 <sup>cd</sup>	-	5.08 <sup>ab</sup>	1.53 <sup>b</sup>	5.18 <sup>b</sup>
T7	$0.22^{ab}$	$6.40^{bc}$	-	5.26 <sup>a</sup>	1.51 <sup>b</sup>	5.14 <sup>c</sup>

a,b Means within the same column with different letters are significantly different  $\left(p{<}0.05\right)$ 

\*Not determined

Figure 1 shows TBARS values during processing. At time 1, referring to mass, there was no statistical difference between results, showing that the formulation used did not immediately affect the oxidation of the raw material. At the end of processing (time 30), it was observed that TBARS values for T7 treatment was statistically higher than the others, followed by T1 and T6. Treatments T2, T3, T4 and T5 were statistically identical to each other, showing the lowest values, which ranged from 0.17 to 0.19 mg malonaldehyde/kg sample; and these values lower than those found by Hoz et al. [6], in dry fermented sausages with the same methodology used for TBARS determination. According to results, it appears that microencapsulated propolis was effective in preventing lipid oxidation in salami samples during processing at 1500 and 2000 ppm concentrations with OSA starch as encapsulating and 1500 and 2500 ppm with arabic gum, since such treatments differed statistically from control, which showed TBARS value of 0.41 mg malonaldehyde/Kg sample. Therefore, OSA starch and arabic gum used as encapsulating agents allowed the release of active material in the middle. It was also observed that 1000 ppm of concentration with OSA starch was insufficient to prevent lipid oxidation, in other words, the content of propolis added was not satisfactory to inhibit the oxidative process. The addition of 4000 ppm of microencapsulated propolis with arabic gum showed pro-oxidant effect, activating components that accelerate the auto-oxidation of lipids. The phenolic compounds present in propolis may have pro-oxidant activity under certain conditions [9], as well as other antioxidants, when applied at high levels in a food system [10].

Ethanolic extract solution of propolis was effective in reducing the lipid oxidation rate in pork cured sausage over 4 weeks of storage [11]. The variations found for TBARS values for the same treatment over time seem to be inherent to the methodology used for quantifying malonaldehyde, which can bind with to proteins and, thus, not be measured even with increasing lipid oxidation rates [12].

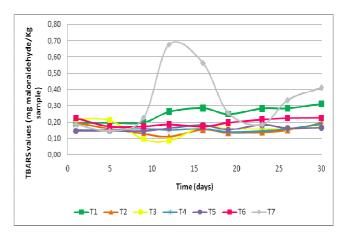


Figure 1. TBARS values (mg malonaldehyde/kg sample) from samples over 30 days of salami processing

Figures 2 and 3 show brightness (L\*) and red intensity (a\*) variation, during salami samples processing, respectively. It was observed that from time 1 to 2, respectively, a reduction and an increase in brightness and red intensity occurred, characterizing the fermentation process and consequent curing process. With the exception of times 19 and 26 for brightness, there was no statistical difference (p<0.05) between treatments during the processing, so that the final product showed L\* from 43.38 to 46.97 and a\* from 15.37 to 17.70. Therefore, it is observed that the addition, the encapsulation material and concentration of propolis microcapsules did not affect significantly L\* and a\*, being possible to be used in meat sausage formulations without affecting the product color.

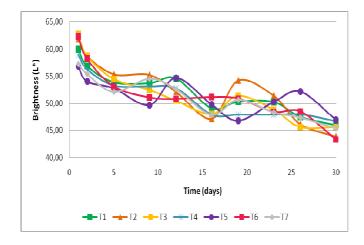


Figure 2. Brightness (L\*) from samples over 30 days of salami processing

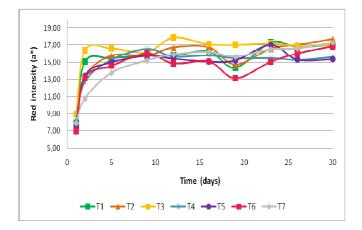


Figure 3. Red intensity (a\*) from samples over 30 days of salami processing

## IV. CONCLUSION

Propolis microencapsulation had great potential for use in dry fermented sausage, which can be extended to other meat products, because it was observed that its use was positive in reducing the lipid oxidation rate without adversely affecting the quality characteristics such as pH, acidity and color of the product throughout its processing.

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#### REFERENCES

- S. Bernardi, M. Oetterer and C. J. Contreras-Castillo. "Embutidos Cárneos: melhoramento no valor nutritivo e aplicação de antioxidantes naturais", *Revista Nacional da Carne*, vol. 32, no. 378, pp. 30-38, Aug. 2008.
- [2] M. C. Marcucci. "Propolis: chemical composition, biological properties and therapeutic activity", *Apidologie*, vol. 26, no. 2, pp. 83-99, 1995.
- [3] G. A. Burdock. "A review of the biological properties and toxicity of bee propolis", *Food and Chemical Toxicology*, vol. 36, no. 4, pp. 347-363, 1998.
- [4] S. Kumazawa, T. Hamasaka and T. Nakayama. "Antioxidant activity of propolis of various geographic origins", *Food Chemistry*, vol. 84, no. 3, pp. 329-339, Feb. 2004.
- [5] C. S. Favaro-Trindade, S. C. Pinho and G. A. Rocha. "Revisão: Microencapsulação de ingredientes alimentícios", Brazilian *Journal of Food Technology*, vol. 11, no. 2, pp. 103-112, Apr./Jun. 2008.
- [6] L. Hoz, M. D'Árrigo, I. Cambero and J. A. Ordóñez. "Development of an n-3 fatty acid and α-tocopherol enriched dry fermented sausage", *Meat Science*, vol. 67, no. 3, pp. 485-495, Jul. 2004.
- [7] M. A. Abastecimento. "Regulamenta métodos analíticos para controle de produtos cárneos e seus ingredientes – Métodos físico-químicos", *Instrução Normativa* no. 20, Jul. 1999.
- [8] D. I. Demeyer, P. Vandekerckhove and R. Moermans. "Compounds determining pH in dry sausage", *Meat Science*, vol. 3, no. 3, pp. 161-167, Jul. 1979.
- [9] E. A. Decker "Phenolics: prooxidants or antioxidants?", *Nutrition Reviews*, vol. 55, no. 11, pp. 396-398, 1997.
- [10] V. C. Ramalho and N. Jorge.. "Antioxidantes utilizados em óleos, gorduras e alimentos gordurosos", *Química Nova*, vol. 29, no. 4, pp. 755-760, Jul./Aug. 2006.
- [11] S. K. Han and H. K. Park. "Accumulation of thiobarbituric acid-reactive substances in cured pork sausages treated with propolis extracts", *Journal of the Science of Food and Agriculture*, vol. 82, pp. 1487-1489, 2002.
- [12] S. L. Melton. "Methodology for following lipid oxidation in muscle foods", *Food Technology*, vol. 37, no. 7, pp. 105-111, 1983.