ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL OF JABUTICABA (*Myrciaria cauliflora*) EXTRACT ADDED TO FRESH SAUSAGES

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Abstract- Jabuticaba extract (*Myrciaria cauliflora*), beyond its desirable reddish color, may show antioxidant and antimicrobial action. The aim of this research was to access the effect of adding Jabuticaba extract regarding microbiological and oxidative stability of fresh sausages. Meat products were prepared with and without the addition of Jabuticaba extract, as follows: Control: without Jabuticaba extract (JE) addition; T2% pork sausage with 2% JE and T4% pork sausage with 4% JE. Products stability during refrigerated storage (1±1ºC), were evaluated by physicochemical (–TBARS Index, pH value and objective color – L*, a* and b* parameters) and microbiological analyses (total counts of aerobic mesophilic and psychotrophic microorganisms, lactic acid bacteria, coliforms at 45ºC, *Staphylococcus aureus* and *Salmonella* sp). Sausages with Jabuticaba extract addition, in both concentrations, showed more desirable reddish color (higher values of a*) and lower lipid oxidation, with T2% and T4% presenting TBARS Index around 0,02 while Control treatment showed 0,27mg MDA/kg sample. In relation to microbiological analyses, the addition of Jabuticaba extract decreased 1 log for mesophilic and psychotrophic microorganisms counts and totally inhibited the growth of coliforms at 45ºC. One can conclude that the addition of Jabuticaba extract improved microbiological and oxidative stability of fresh pork sausage.

I. INTRODUCTION

As many others food products, fresh sausage is processed in some way before reaching the consumers and could lose its desirable color along processing and storage. In this sense, manufactures have the necessity to replace colors lost during food processing or also to increase the color of products that would be colorless (2) or show unpleasant appearance. Currently, it has been highlighted using natural dyes that, beyond the purpose of food coloring may also have antioxidant and antimicrobial action. Most agricultural waste generated during processing is discarded without treatment, causing harm to the environment. Many of these residues are rich in bioactive compounds, able to fight oxidative damage caused by free radicals, such as antioxidants, substances of high commercial value (3). The waste generated from Jabuticaba (*Myrciaria cauliflora*) processing into jam and candys is rich in anthocyanins (phenolic compounds called flavonoids, present in the fruit skins and seeds) and can be used as natural dyes still presenting antioxidants and antimicrobials effects (4). In meat products, one of the biggest problems encountered due to processing and storage is lipid oxidation, negatively affecting the color, flavor and nutritional value of the product (5). In order to inhibit the development of these oxidative reactions, natural antioxidants have been used in the meat industry while maintaining the quality, prolonging the shelf life and ensuring food safety (6). Another problem related to fresh sausage is microbial deterioration because been a non-thermally treated product usually presents high microbial load. In this regard, the objective of this study was to evaluate the antimicrobial and antioxidant potential of Jabuticaba extract added to fresh sausage, evaluating its stability during refrigerated (1±1ºC) storage.

II. MATERIALS AND METHODS

To obtain the extract of Jabuticaba its pulping was conducted to give the residue (peels and

seeds) and Jabuticaba pigment extraction was performed with adding water at a ratio of 1:3 (residue: water), in the absence of light under mechanical stirring (Agitator Marconi) for 6 hours. The fluid obtained was filtered and the crude extract was concentrated to 1/3 of its original volume using a rotary evaporator (Tecnal, TE-211) at 60°C coupled to a vacuum pump (Tecnal, TE-058). After concentration, the extract was dryed in a spray dryer using maltodextrin as a carrier agent. After these procedures, the dry Jabuticaba extract was applied to fresh sausage. Three differents treatments were performed, which formulations are described in Table 1.

Table 1. Composition of different treatments for fresh sausage

	Quantities (%)		
	Control	T 2%	T 4%
Pork shoulder	70	70	70
Pork backfat	25	23	21
Water	3	3	3
Sodium chloride	1	1	1
Condiment	1	1	1
Phosphate	0,25	0,25	0,25
Jabuticaba Extract	0	2	4

Control: without jabuticaba extract, T 2%: 2% jabuticaba extract, T 4%: 4% jabuticaba extract.

Three kilograms of fresh sausage have been processed, being one kilogram of each different treatment. The fresh sausages were processed according to Normative Instruction n°. 4/2000 (BRAZIL, 2000), packaged in permeable plastic bag (without vacuum) and stored in an environmental chamber at 1±1°C for 15 days. Tests for evaluation of oxidative and microbiological stability of the fresh sausages were performed as described below. For the analysis of thiobarbituric acid reactive substances (TBARS) was used the method described by Hodges et al. (8) suitable for evaluation of lipid oxidation of materials containing colored compounds, such as anthocyanins. For measurements of pH values a pH meter (Model HI 99163, HANNA Brand) with combined electrode was applied for reading in triplicate and color analysis was carried out using a portable colorimeter (mod. MiniScan XE HunterLab mark) through the scale L *, a *, b *, CIE Lab system. Microbiological counts of mesophilic and psychrotrophic and lactic acid bacteria (9) were applied for evaluation of spoilage microorganisms and coliforms at 45°C (AOAC No. 989.10), *Staphylococcus aureus* (AOAC No. 2003.11) and *Salmonella* sp were performed (AOAC 2003.09) as indicators of pathogenics (10).

III. RESULTS AND DISCUSSION

The fresh sausages were analyzed for thiobarbituric acid reactive substances (TBARS), pH values, objective color and microbiological parameters after 15 days of storage. The results obtained for lipid oxidation (TBARS) are described in Table 2.

Table 2. Results of lipid oxidation (TBARS[a]) of fresh sausage stored at 1±1°C for 15 days.

Treatments	mg MDA/kg[b]
Control	0,27±0,023[c]
T 2%	0,02±0,005
T 4%	0,01±0,012

Control: without jabuticaba extract, T 2%: 2% jabuticaba extract, T 4%: 4% jabuticaba extract
[a]TBARS = Thiobarbituric acid reactive substances
[b]malonaldehyde milligram per kilogram of sample
[c]Values expressed as means ± standard error
For each treatment were used two pieces of fresh sausages (250g)

Control sample has showed a higher rate of TBARS than the sausages made with two levels of Jabuticaba extract. Thus, one may indicate that anthocyanins present in the Jabuticaba extract were effective in reducing lipid oxidation. These results are similar to those found by Salem & Ibrahim (11), who have studied the addition of sage oil extract at concentrations of 0.05 and 0.025% in sausage made with buffalo meat, and achieved values of 0.91 for treatment without natural antioxidant and 0.51 and 0.46 mg MDA/kg of product for treatments with the extract of sage. Jayawardana et al. (12), used Adzuki beans in the concentration of 0,2% in cured and uncured sausages and their use controlled effectively lipid oxidation in both types of sausages, and this reduction can be attributed to the presence of polyphenols, which are rich in anthocyanins present in Adzuki bean extract. The average results of pH values are represented in Table 3.

Table 3. Results of pH values of fresh sausage stored at 1±1°C for 15 days.

Treatments	pH (Mean±SE[a])
Control	6,43±0,015
T 2%	5,47±0,010
T 4%	5,36±0,038

Control: without jabuticaba extract, T 2%: 2% jabuticaba extract, T 4%: 4% jabuticaba extract.
[a]Values expressed as means ± standard error.
For each treatment were used two pieces of fresh sausages (250g)

According to the results there was a reduction in the pH values of the fresh sausages as there was an increase in the amount of added extract which can be explained by the lower pH of the Jabuticaba extract (3,7) which probably led to a reduction of the pH values in the sausages. These acidic conditions can provide a favorable environment for growth of lactic acid bacteria (13). In the work by Ibrahim & Salem (10) there was a decrease in pH from 6.9 to 4.5 in the first two days and the production of organic acids by bacteria may be the cause for the observed decrease.
The results obtained in the determination of objective color are described in Table 4.

Table 4. Results of objective color analysis of fresh sausage stored at 1±1°C for 15 days.

Treatments	L*	a*	b*
Control	64,88±2,51[a]	1,56±0,46	12,68±0,94
T 2%	59,99±0,95	4,11±0,19	7,95±0,45
T 4%	60,12±0,68	4,75±0,24	5,45±0,35

Control: without jabuticaba extract, T 2%: 2% jabuticaba extract, T 4%: 4% jabuticaba extract.
[a]Values expressed as means ± standard error.
For each treatment were used two pieces of fresh sausages (250g)

The color decisively influences in consumer preference when purchasing a food. The visual impact of the color generally overlaps that caused by other attributes (14). According to the results for the values of L*, a*, b*, treatments using Jabuticaba extract preserved meat pigments against the occurrence of oxidation checked by the highest and lowest a* b* values. Kim et al. (15) found that the use of 0.1 and 0.5% extract of broccoli leaves and cocklebur (containing phenolic compounds) in ground beef decreased L* and b* over the period of storage. The value of a *, which is the most important parameter in assessing the oxidation of the meat also decreased with storage, therefore the control treatment without the natural antioxidant has a faster reduction in the value of the a* and at the end of storage this value in meat products containing antioxidants was greater than the control sample.
For the analysis of microorganisms (Table 5) we can verify that the treatments with Jabuticaba extract showed a reduction of 1 log in the aerobic mesophilic and psychotrophic microorganisms counts and also that the extract addition at 2 and 4% totally inhibited the growth of coliforms at 45°C. Similar results was reported by Liu et al. (17) who observed lower counts of aerobic mesophilic microorganisms in fresh sausage with addition of rosemary. Busatta et al. (18) evaluated the antimicrobial activity of essential oil of marjoram applied in fresh sausage and verified bacteriostatic effect on *Escherichia coli,* beyond a reduction in total count of microorganisms. For *Staphylococcus aureus* there was no colony count for all treatments. In contrast, Erkmen (19) found that the addition of *Thymbra spicata* essential oil resulted in a reduction in the count of *S. aureus* in 1.74 log, compared to the control treatment. Moreover, the same author reports 1.04 log reduction in total microorganisms.
For the presence of *Salmonella*, treatments with and without the natural antioxidant are within the recommended legislation by RDC 12/2001 (16) advocating absence in 25g of sample. On the other hand, for the lactic acid bacteria there was an increase of about 1 log for the treatments with the Jabuticaba extract (*Myrciaria cauliflora*) when compared to Control. This could be explained by the favorable low pH value found in these sausages and also by the use of maltodextrin in microencapsulation of extract which served as substrate for the growth of these microorganisms.

Table 5. Results of microbiological analysis (Log CFU.g−1/sample) of fresh sausage stored at 1±1°C for 15 days

Microorganisms	Counts (log CFU.g−1[a])		
	Control	T 2%	T 4%
Coliforms at 45°C	1,60	0	0
Aerobic Mesophilic	6,80	5,80	5,94
Aerobic Psychrotrophic	7,74	6,13	6,61
Lactic Acid bacteria	6,96	7,54	7,57
Salmonella sp	Aus[b]	Aus	Aus

Control: without jabuticaba extract, T 2%: 2% jabuticaba extract, T 4%: 4% jabuticaba extract.
[a]CFU·g−1 = colony forming units per gram.
[b]Absence in 25g of sample
For each treatment were used two pieces of fresh sausages (250g)

IV. CONCLUSION

With the results, it can be confirmed that the use of Jabuticaba extract (*Myrciaria cauliflora*), added to the fresh sausage reduced lipid oxidation and decreased counts of microorganisms in the product.

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