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Natural antioxidant from Pequi (*Caryocar brasiliense* Camb.) peel in the production of sausage

[1*]Monteiro, S. S., [2]Copetti, C., [2]Nogara, G., [2]Dalla Nora, F. M., [3]Prestes, R. C. and [3]da Rosa, C. S.

[1]*Nutrition Course, Institute of Health and Biotechnology, Federal University of Amazonas – UFAM, Amazonas, Brazil*
[2]*Federal University of Santa Maria – UFSM, Rio Grande do Sul, Brazil*
[3]*Departament of Science and Food Technology, Centre for Rural Sciences, Federal University of Santa Maria – UFSM, Rio Grande do Sul, Brazil*

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Abstract

The present study aimed to test different concentrations (0.5%, 1.0% and 1.5%) of extract of pequi (*Caryocar brasilense* Camb.) peel in chicken sausage and analyze the chemical composition, colour, effect of the extracts on lipid stability, microbiological composition and sensory attributes during storage. The chemical composition met the standards of identity and quality. The sausages showed darkening during storage. At the end of the storage period, the TBARS values for the sausage with 1.5% extract were 0.421 ± 0.10 mg MDA.Kg^{-1} sample and 1.375 ± 0.11 mg MDA.Kg^{-1} for the control sample. The microbiological analysis showed that the chicken sausages were within the limits prescribed by law. The acceptability index for the sensory attributes was good for the sausages with up to 1.0% of pequi peel extract after six days of storage. The results of the intent to purchase test conducted at six days of storage showed that the sausage with 1.0% added extract of pequi peel was preferred.

Introduction

Lipid oxidation is a factor that limits the quality of meat and meat products and interferes with consumer acceptability. This phenomenon causes commercial loss and means that the meat products industry has to adopt measures that limit its effects because the oxidation process involves organoleptic changes in products, such as changes in the colour of the meat and fat, and the development of an unpleasant taste and aroma that make the food unfit for consumption, as well as causing other changes that affect nutritional quality through the formation of potentially toxic, mutagenic and carcinogenic compounds (Mariutti and Bragagnolo, 2007).

Lipid oxidation is normally associated with cooked meat, or meat whose muscle membranes undergo a disintegration process, such as the grinding of raw material which occurs in the preparation of sausages. In the case of chicken sausages, the onset of the oxidative rancidity reaction is catalysed by the action of the oxygen in the air on the unsaturated fatty acids present in the fat of the chicken (Mariutti and Bragagnolo, 2009). Antioxidants are used to prevent or slow down lipid oxidation in products. However, the addition of synthetic antioxidants began to be curtailed in recent years, due to the decrease in consumer acceptance and harmful effects to human health (Martha-Estrella *et al*., 2007).

Several studies have been conducted to verify the antioxidant potential of phenolic compounds, in order to replace synthetic antioxidants by natural antioxidants in the prevention of lipid oxidation and to participate in the processes responsible for the colour, astringency and aroma in various foods (Pokorny *et al*., 2001). Pequi is the popular name of *Caryocar brasiliense* Camb. of the *Caryocaceae* family. It is aslo known as piqui, pequiá, amêndoa de espinho, grão de cavalo and amêndoa do Brasil. The trees are found in hot regions in the north and centre-west of Brazil, and it is a fruit that is typical of the Cerrado (Gonçalves *et al.*, 2011). The fruit is a drupe, composed of 76.7% peel, 21.6% of seed (kernels) and 1.7% of fruitlets (kernels that have not completed their physiological development). According to Marques (2001), the peel is composed of two layers: one is thin, leathery, grayish green (exocarp) and the other is thick and fleshy, yellowish white (outer mesocarp). Pequi peel is rich in phenolic compounds and it has compounds that protect the lipid fraction from oxidation.

This study aimed to test different concentrations (0.5%, 1.0% and 1.5%) of extract of pequi (*Caryocar brasilense* Camb.) peel In chicken sausage, characterising chemical composition, colour, pH, assessment of the effect of the extracts on lipid and

*Corresponding author.
Email: *claudiasr37@yahoo.com.br*
Tel: +55 55 3220 8254

microbiological stability, and sensory attributes during storage.

Materials and Methods

Raw material

Chicken meat and other ingredients

The chicken meat (deboned thigh and drumstick with skin) was donated by the Central Aurora Foods Cooperative company (Quilombo, SC, Brazil). The other ingredients used in the formulation of the sausages were purchased from commercial establishments in the municipality of Santa Maria, Rio Grande do Sul.

Pequi peel for preparation of extracts

Ripe fruits were purchased from the Grande Sertão Cooperative (Montes Claros, MG, Brazil) in January 2012 and transported in plane coolers. After receipt they were selected for absence of defects, pests and diseases; they had their surfaces washed with mild detergent to remove dirt and were rinsed in running water. Then, sanitisation was performed with 200 $mg.L^{-1}$ sodium hypochlorite for 20 min and then they were cut manually, in a diametrical direction, with stainless steel knives to separate the peel from the seeds. Afterwards, the peel was subjected to bleaching in water at 75°C for 6 minutes, and immediately immersed in iced water, vacuum packaged and stored at -18°C until use.

For the extraction, the peel was dried in an oven with forced air circulation at 55°C for 48 hours. Then the sample was milled in an analytical mill refrigerated at 4°C (Quimis, model Q 298A21, Brazil) using an ultra-thermostat bath (Solab, model SL-152/10, Brazil) and it was then then standardised into a particle size of 60 mesh (0.25 mm). For extraction, the peel was dried in an oven with forced air circulation at 55°C for 48 hours.

Obtaining the extract of pequi peel

For the extraction by shaking, the methodology was as described by Lima (2008) with modifications. The extract was prepared from the previously milled pequi peel, weighed (1 g) in a beaker and added to 50 ml of solvent (80% hydroethanolic). Then the mixture was brought to an ultra-thermostat bath (Solab model SL-152/10, Brazil) and subjected to constant stirring using a shaker (Marconi MA-039 Brazil) for 20 minutes at a temperature of 70°C. Afterwards, the extracts were filtered through a paper filter and centrifuged at 3000 rpm for 20 minutes. The filtrate was concentrated to 20% of its initial volume in a rotary evaporator (Fisaton 802, Brazil) with 7060 mg Hg vacuum and water bath at a temperature of 40°C (± 1°C). The extract was stored in amber bottles and stored in a freezer (-18°C) prior to use. Extraction by shaking was performed in the laboratory of the Department of Food Science and Technology at the Federal University of Santa Maria, RS.

Table 1. Base formulation of chicken sausage used in the treatments

Raw materials and ingredients	Quantity (%)
Thigh and drumstick*	88.674 - 90.074
Water (only formulation)	3.000
Chicken seasoning	0.400
Carrageenan	0.300
Liquid carmine dye	0.010
Textured soy protein	2.500
Sugar	0.300
Refined salt	2.200
Sodium nitrite	0.015
Monosodium glutamate	0.100
Sodium erythorbate**	0.100
Extract of pequi peel***	0.500 - 1.500
Sodium tripolyphosphate	0.500
White pepper	0.001
Garlic powder	0.500

*Varied according to treatment. **Only in the control treatment. ***Except for the control treatment.

Preparation of the product

For the preparation of the chicken sausages the ingredients and requirements outlined by the legislation (Brasil, 2000) and procedures described by Land (1998) were taken into consideration (Table 1). The chicken sausage was prepared from ground chicken meat (Jamar PJ22 grinder, Jamar Ltda, Brazil) using a disk with a diameter of 10 mm and it was transported to the mixing machine (Jamar MJI 35, Brazil) to receive the remaining ingredients and then mixed to obtain binding. The addition of 80% hydroethanolic extract of pequi peel (EPP) was performed manually at the end of the formulation, except for the control treatment, which did receive the addition of the extract. The treatments were as follows:

Control – without the addition of the extract (C); T1 – 0.5% extract of pequi peel (0.5% EPP); T2 – 1.0% extract of pequi peel (1.0% EPP); and T3 – 1.5% extract of pequi peel (1.5% EPP).

The meat mixtures were embedded in swine guts cleaned with acetic acid and tied into segments with a characteristic size (10 cm) and then packaged and identified. For storage, the sausages were packed in polystyrene trays, wrapped with plastic wrap, identified and stored at +4°C (± 1ºC).

Chemical composition of the product

The chemical composition was performed on day 1 of storage; the samples were crushed in a

multiprocessor to form a smooth paste. Analyses of moisture, ash and crude protein were performed in accordance with the official methods (AOAC, 1998). The lipids were determined by the butirometric method, which is based on the selective attack of organic matter by sulfuric acid, with the exception of the fat, which is separated by centrifugation aided by amyl alcohol, which modifies the surface tension according to Terra and Brum (1988).

pH determination

The pH readings were performed on days 1, 7, 14, 21, 28, 35 and 42 after the manufacture of the product. Ten grams of sample were homogenised with distilled water (1:10 sample/water) in a blender. The electrodes of the Digimed pH meter were introduced into the homogenised material for 5 minutes and readings were in triplicate (Terra and Brum, 1988).

Colour determination

The colour was measured using a calibrated colorimeter (Minolta Chroma Meter CR-300 Brazil) and measured on days 1, 7, 14, 21, 28, 35 and 42 after manufacture. The mixture was removed from the casing and then homogenised and distributed in Petri dishes. The results were expressed as L^*, which represents the percentage of light ranging from black (0%) to white (100%); a^*, where $-a^*$ represents direction to green, and $+a^*$ represents direction to red; b^* where $-b^*$ represents direction to blue and $+b^*$ represents direction to yellow; C^* (saturation index) and h^* (hue angle). For each treatment an average value of five readings at different points on the surface was obtained (Ramos and Gomide, 2007).

Lipid oxidation

To assess the extent of lipid oxidation occurring in the sausages the test of substances reactive to 2-thiobarbituric acid (TBARS) was performed according Raharjo *et al.* (1992), modified by Wang *et al*. (2002), in relation to the interference of sugar in the reaction and following the recommendations of Shaidi *et al.* (1985) with regard to the addition of sulfanilamide for samples containing nitrite. 10 g of previously ground and homogenised sample was weighed in a plastic sachet and 40 mL of 5% trichloroacetic acid (TCA) and 1 mL of 0.15% synthetic butylated hydroxytoluene (BHT) antioxidant was added. This was homogenised in a Stomacher for 1 minute and filtered with the aid of qualitative filter paper into a 50 mL volumetric flask and the volume was completed with a solution of 5% trichloroacetic acid. From this balloon, a 5mL aliquot was withdrawn and transferred to a test tube, where 5 mL of 0.08 M thiobarbituric acid in 50% acetic acid was added. The tubes were incubated in a boiling water bath for 40 minutes.

The concentration was estimated by spectrophotometry (Parkin Elmer, Lambada EZ150 model, Brazil) at 531 nm using a standard curve with 2-thiobarbituric acid (1×10^{-8} at 1×10^{-7} mol. mL^{-1}). Results were expressed as milligrams of malonaldehyde per kilogram of sample (mg MDA. Kg^{-1} of sample). The determination of lipid oxidation was performed on days 1, 7, 14, 21, 28, 35 and 42 after manufacture.

Microbiological analyses

The analyses of positive and negative *Staphylococcus coagulase*, coliform count at 35ºC and 45ºC, *Salmonella* sp and *Clostridium sulfito* were performed on day 1 only (Brasil, 2003). The mesophilic and psychrotrophic analyses were performed on days 1, 7, 14, 21, 28, 35 of product storage at 4°C (± 1°C) (APHA, 2001).

Sensory analysis

An affective acceptability test was conducted with 50 untrained testers using a seven-point hedonic scale (1 = extremely dislike and 7 = extremely like) as in the recommended methodology (IAL, 2008), with some adaptations. The evaluation was performed on days 6 and 20. The attributes evaluated were colour, odour, taste, texture and appearance.

The evaluation was conducted in individual booths in the sensory analysis laboratory of the Department of Food Science and Technology, at the Centre for Rural Sciences, UFSM, in the morning between 9 and 11 hours and planned so that each participant tasted the 4 samples that were served sequentially in completely balanced blocks with respect to the order of presentation.

The chicken sausages were roasted for 45 minutes at 180°C. They were then sliced, and a slice of each treatment was served on white paper plates, properly identified with three-digit random numbers and presented in a monodic form to the testers. Each tester was also given a glass of water to clean the taste buds. The purchase intention test was also performed, by which the testers expressed their willingness to consume, acquire or buy a product. A structured five-point scale was used (1 = would certainly not buy and 5 = would certainly buy) (Meilgaard *et al*., 1987).

For the calculation of the product acceptability index the following expression was adopted: IA (%) = A x 100 / B, where A = average grade obtained for the product and B = maximum grade given to the product. A good IA is considered to be ≥ 70%

(Dutcosky, 2011).

Statistical analysis

Results were expressed as mean ± standard deviation and subjected to analysis of variance (ANOVA) and the means were compared using Tukey's test with a significance level of 95% ($p < 0.05$). The results were analysed using SPSS version 19.0.

Results and Discussion

Chemical composition of chicken sausage

The results of the chemical composition of chicken sausages with added extract of pequi peel and the control can be seen in Table 2. The moisture, protein, ash and fat contents were not significantly different ($p > 0.05$) between treatments. These results were in accordance with the Standard of Identity and Quality (Brasil, 2000), which establishes the maximum value of 70% for moisture, 30% for lipids and a minimum value of 12% for protein.

pH

The pH results are shown in Table 3. The pH values were in the range between 5.90 and 6.68 in the different analysed treatments, showing significant difference ($p < 0.05$) between treatments on days 1, 28 and 42. The results were within the values reported by Olivo (2006), because the normal pH values for chicken thigh are 6.40 and 6.70, and for breast meat 5.94. For manually deboned drumstick meat, a pH of about 5.80 to 6.20 is considered normal.

During the period of storage there was a reduction in pH. A decrease in pH was also reported by Almeida (2005) in Tuscan sausage, after 10 days of storage at 4 °C, packaged in oxygen permeable film, where the pH decreased from 5.88 to 5.68. This fact may possibly indicate that fermentation occurred during storage due to the glucose contained in the rapid curing used in that study; as well as the glucose contained in the rapid curing there was also added sugar in the formulation of the sausage, which leads to fermentation and decay of pH.

The pH of frescal chicken sausage is a variable that depends on many factors such as the state of conservation of the sausage and its microbiological conditions. The pH of a food not only exerts influence on the rate of multiplication of micro-organisms, but also interferes with the quality of food during storage, heat treatment, drying, or during any other type of treatment; it is also directly responsible for the deterioration of food products (Silva, 2000).

Table 2. Chemical composition of samples of chicken sausage; control and with different added concentrations of extract of pequi (*Caryocar brasiliense* Camb.) peel during storage at 4°C (± 1 ºC)

	Chemical Composition			
Fractions g (%)	Control	0.5% EPP*	1.0% EPP	1.5% EPP
Moisture	$61.97^{a} \pm 0.697$	$62.90^{a} \pm 0.917$	$62.57^{a} \pm 0.211$	$62.90^{a} \pm 0.372$
Protein	$19.17^{a} \pm 0.976$	$18.78^{a} \pm 0.886$	$18.68^{a} \pm 0.833$	$19.35^{a} \pm 0.817$
Ash	$4.55^{a} \pm 0.405$	$4.56^{a} \pm 0.115$	$4.55^{a} \pm 0.203$	$4.65^{a} \pm 0.311$
Fat	$11.67^{a} \pm 0.930$	$11.38^{a} \pm 0.646$	$11.56^{a} \pm 0.368$	$11.13^{a} \pm 0.408$
Carbohydrates	$2.64^{a} \pm 0.471$	$2.39^{a} \pm 0.567$	$2.64^{a} \pm 0.895$	$1.97^{a} \pm 0.461$

Values presented as mean ± standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$) by Tukey's test.
*EPP: Extract of pequi peel.

Table 3. Average pH values of the samples of chicken sausage; control and with different added concentrations of extract of pequi (*Caryocar brasiliense* Camb.) peel during storage at 4ºC (±1ºC)

	pH			
Days of storage	Control	0.5% EPP*	1.0% EPP	1.5% EPP
1	$6.44^{b} \pm 0.045$	$6.61^{a} \pm 0.003$	$6.45^{b} \pm 0.036$	$6.51^{ab} \pm 0.060$
7	$6.57^{a} \pm 0.060$	$6.60^{a} \pm 0.074$	$6.58^{a} \pm 0.111$	$6.64^{a} \pm 0.028$
14	$6.46^{a} \pm 0.034$	$6.51^{a} \pm 0.034$	$6.53^{a} \pm 0.257$	$6.51^{a} \pm 0.234$
21	$6.50^{a} \pm 0.090$	$6.68^{a} \pm 0.112$	$6.60^{a} \pm 0.037$	$6.62^{a} \pm 0.095$
28	$6.34^{b} \pm 0.066$	$6.39^{ab} \pm 0.049$	$6.49^{a} \pm 0.054$	$6.44^{ab} \pm 0.066$
35	$6.28^{a} \pm 0.076$	$6.41^{a} \pm 0.083$	$6.38^{a} \pm 0.071$	$6.38^{a} \pm 0.032$
42	$6.25^{a} \pm 0.020$	$6.31^{a} \pm 0.039$	$5.90^{b} \pm 0.025$	$6.28^{a} \pm 0.022$

Values presented as mean ± standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$) by Tukey's test.
*EPP: Extract of pequi peel.

Colour determination

The results obtained for luminosity (L^*), redness (a^*), yellow (b^*), saturation index (C^*) and hue angle (h^*) of the chicken sausage during storage are shown in Table 4. For the L^* parameter it was observed that from the fourteenth day of storage there was no significant difference ($p < 0.05$) between the control and the other treatments tending to black (0%) with the addition of different concentrations of pequi peel extract. At the end of storage all the treatments showed a decrease in the L^* values relative to the start of the experiment, indicating a darkening of the product.

Regarding the a^* parameter, until the twenty-first day of storage, there was a significant difference ($p < 0.05$) between the control and the other treatments, and from day 28 to day 35 the treatment with the highest concentration of extract (1.5%) was significantly equal ($p > 0.05$) to the control. Furthermore, there was an increase in values found at the end of storage compared to the beginning, with high values for the a^* parameter, related to the concentration of myoglobin and nitrosomioglobina formation during the curing process. This tendency to red may also be due to the addition of carmine dye in the formulation of chicken sausages.

The b^* parameter, ranging from blue ($-b^*$) to yellow ($+b^*$) showed higher values for the control throughout the storage period and significant difference ($p < 0.05$) compared with the other treatments. The tendency to yellow displayed by the control possibly occurred

Table 4. Mean values for luminosity (L^*), parameter (a^*), parameter (b^*), saturation index (C^*) and hue angle (h^*) of chicken sausage; control and different added concentrations of extract of pequi (*Caryocar brasiliense* Camb.) peel during storage at 4°C (± 1°C)

		*L** parameter		
Days of storage	Control	0.5% EPP*	1.0% EPP	1.5% EPP
1	60.56^{a} ± 1.332	58.45^{ab} ± 1.564	58.57^{ab} ± 0.987	58.20^{b} ± 0.655
7	56.49^{a} ± 0.499	52.03^{c} ± 0.543	54.41^{b} ± 0.912	56.28^{a} ± 0.615
14	54.57^{a} ± 0.638	51.87^{b} ± 1.292	52.89^{b} ± 0.452	52.53^{b} ± 1.059
21	55.92^{a} ± 1.521	52.07^{b} ± 1.075	50.80^{b} ± 0.854	52.03^{b} ± 0.826
28	54.94^{a} ± 0.986	51.50^{b} ± 0.821	50.33^{b} ± 0.695	50.98^{ab} ± 1.072
35	55.97^{a} ± 0.507	51.06^{b} ± 1.597	50.10^{b} ± 1.260	50.17^{b} ± 1.149
42	54.44^{a} ± 1.242	48.79^{b} ± 0.942	49.68^{b} ± 0.923	49.26^{b} ± 1.092
		*a** parameter		
Days of storage	Control	0.5% EPP	1.0% EPP	1.5% EPP
1	16.23^{a} ± 0.470	12.59^{b} ± 0.538	11.78^{b} ± 0.198	11.81^{b} ± 0.523
7	16.29^{a} ± 0.536	10.80^{c} ± 0.179	12.31^{b} ± 0.279	12.79^{b} ± 0.223
14	15.46^{a} ± 0.362	9.15^{d} ± 0.437	11.00^{c} ± 0.350	13.10^{b} ± 0.179
21	12.49^{a} ± 0.582	8.71^{d} ± 0.418	10.37^{c} ± 0.270	11.33^{b} ± 0.276
28	13.01^{a} ± 0.803	10.45^{b} ± 0.762	11.19^{b} ± 0.833	13.60^{a} ± 0.736
35	14.77^{a} ± 0.553	11.02^{b} ± 0.597	11.66^{b} ± 0.665	15.05^{a} ± 0.142
42	16.45^{a} ± 1.378	13.11^{c} ± 0.244	14.88^{ab} ± 0.669	15.80^{ab} ± 0.294
		*b** parameter		
Days of storage	Control	0.5% EPP	1.0% EPP	1.5% EPP
1	17.10^{a} ± 0.544	15.00^{b} ± 0.519	14.62^{bc} ± 0.275	14.19^{c} ± 0.298
7	16.28^{a} ± 0.370	13.32^{b} ± 0.328	13.64^{b} ± 0.556	13.92^{b} ± 0.381
14	15.87^{a} ± 0.243	12.95^{bc} ± 0.246	12.76^{c} ± 0.340	13.38^{b} ± 0.302
21	16.41^{a} ± 1.006	13.12^{b} ± 0.431	12.48^{b} ± 0.428	13.08^{b} ± 0.589
28	16.53^{a} ± 0.822	12.47^{b} ± 0.869	12.35^{b} ± 0.691	13.00^{b} ± 0.483
35	17.00^{a} ± 0.550	12.83^{b} ± 0.464	13.21^{b} ± 0.342	12.90^{b} ± 0.299
42	16.90^{a} ± 0.538	12.02^{b} ± 0.307	12.03^{b} ± 0.254	12.63^{b} ± 0.389
		*C** parameter		
Days of storage	Control	0.5% EPP	1.0% EPP	1.5% EPP
1	23.57^{a} ± 0.681	19.45^{b} ± 0.680	18.77^{bc} ± 0.302	18.51^{c} ± 0.210
7	22.97^{a} ± 0.639	17.14^{c} ± 0.348	18.37^{b} ± 0.554	18.90^{b} ± 0.405
14	22.15^{a} ± 0.390	15.85^{d} ± 0.405	16.85^{c} ± 0.407	18.72^{b} ± 0.259
21	20.63^{a} ± 0.731	15.75^{c} ± 0.303	16.22^{c} ± 0.346	17.31^{b} ± 0.436
28	21.61^{a} ± 0.590	16.02^{c} ± 0.504	16.59^{c} ± 0.454	18.00^{b} ± 0.850
35	22.52^{a} ± 0.698	16.92^{c} ± 0.522	17.62^{c} ± 0.472	19.81^{b} ± 0.287
42	23.59^{a} ± 1.266	17.79^{c} ± 0.267	19.13^{bc} ± 0.669	20.23^{b} ± 0.403
		*h** parameter		
Days of storage	Control	0.5% EPP	1.0% EPP	1.5% EPP
1	46.58^{b} ± 0.576	50.48^{a} ± 0.923	51.18^{a} ± 0.466	50.08^{a} ± 1.299
7	45.04^{c} ± 0.445	51.06^{a} ± 0.534	48.02^{b} ± 0.847	47.46^{b} ± 0.537
14	45.80^{c} ± 0.534	54.84^{a} ± 1.071	49.30^{b} ± 0.922	45.64^{c} ± 0.744
21	53.54^{a} ± 1.847	56.48^{a} ± 1.874	50.30^{b} ± 1.351	49.14^{b} ± 1.584
28	51.02^{b} ± 0.904	53.82^{a} ± 0.960	48.96^{c} ± 0.876	45.68^{d} ± 0.554
35	49.08^{a} ± 0.920	49.38^{a} ± 1.830	48.66^{a} ± 1.872	40.52^{b} ± 0.492
42	45.86^{a} ± 1.882	42.46^{b} ± 0.921	38.90^{c} ± 0.765	38.56^{c} ± 0.789

Values presented as mean ± standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$) by Tukey's test.
*EPP: Extract of pequi peel.

because oxidation was always higher from the beginning of the storage period. According to García-Esteban *et al.* (2004), differences in b^* values during the storage period may be related to the intensity of the oxidation process which appears during storage and which tends to enhance the yellow colour of rancid products.

The degree of saturation (C^*) and hue angle (h) are measurements derived from a^* and b^* and it can be seen that for C^* values, during the entire period of storage, the control was significantly different ($p < 0.05$), with higher values than the treatments; being values closest to the colour gray because the chroma values closest to zero represent neutral colours (grays), while values close to 60 express vivid colours (Mendonça *et al*., 2003).

The hue angle (h^*) is the quantity associated with wavelengths of the visible spectrum, representing the quality of the colour (blue, red, yellow, etc.) and allowing differentiation (Ramos and Gomide, 2007). Studying Table 4, it can be seen that on the first day of storage the treatments with added extract showed higher values and significant difference ($p < 0.05$) compared to control, and after 42 days of storage there was a reversal when control started to present values significantly higher than the other treatments.

Table 5. Mean TBARS values (mg MDA.Kg^{-1} of sample) of samples of chicken sausage; control and with different added concentrations of extract of pequi (*Caryocar brasiliense* Camb.) peel during storage at 4ºC (±1ºC)

		TBA		
Days of storage	Control	0.5% EPP*	1.0% EPP	1.5% EPP
1	^{BC}0.589^{a} ± 0.36	^{B}0.320^{a} ± 0.15	^{A}0.402^{a} ± 0.10	^{BC}0.378^{a} ± 0.21
7	^{C}0.408^{a} ± 0.26	^{B}0.409^{a} ± 0.04	^{A}0.323^{ab} ± 0.04	^{C}0.168^{b} ± 0.06
14	^{BC}0.487^{ab} ± 0.09	^{B}0.357^{b} ± 0.27	^{A}0.645^{ab} ± 0.05	^{A}0.666^{a} ± 0.08
21	^{BC}0.564^{a} ± 0.07	^{AB}0.595^{a} ± 0.02	^{A}0.650^{a} ± 0.06	^{AB}0.587^{a} ± 0.06
28	^{B}0.897^{a} ± 0.18	^{AB}0.595^{b} ± 0.18	^{A}0.407^{b} ± 0.17	^{AB}0.608^{ab} ± 0.07
35	^{AB}0.902^{a} ± 0.08	^{A}0.892^{a} ± 0.06	^{A}0.530^{a} ± 0.31	^{AB}0.548^{a} ± 0.20
42	^{A}1.375^{a} ± 0.11	^{A}0.847^{b} ± 0.12	^{A}0.445^{c} ± 0.12	^{ABC}0.421^{c} ± 0.10

Values presented as mean ± standard deviation. Different small letters in the same row indicate significant difference ($p < 0.05$), Tukey's test. Different capital letters in the same column indicate significant difference ($p < 0.05$), Tukey's test.
*EPP: Extract of pequi peel.

Lipid oxidation (TBARS)

The TBARS results are shown in Table 5. It can be seen that, in general, the TBARS levels tended to increase during the storage period. Several authors who have studied the development of oxidative rancidity claim that it even occurs during storage of frozen chicken, because although deteriorative reactions (microbiological and enzymatic) can be inhibited with the use of low temperatures, lipid oxidation still occurs normally, although at a reduced speed (Grau *et al.*, 2000; Gomes *et al.*, 2003; Brannan, 2008).

On day 1, there was no significant difference between the control and the treatments ($p > 0.05$). From day 7, samples treated with the extract showed significant differences ($p < 0.05$) compared to control. It was also observed that the higher the concentration of the pequi peel extract (1.5%), the greater the inhibition of lipid oxidation at the end of the storage period of the chicken sausages. Rancid odors could be detected by trained and untrained testers in the range 0.5-1.0 and 0.6-2.0 mg MDA. Kg^{-1} sample, respectively. The sausages remained imperceptible to rancid aroma after 6 days of storage, and after 21 days of storage it was noted that in all treatments there was a reduction in the rate of acceptance by the panel of testers, with TBARS levels of about 0.6 mg MDA. Kg^{-1} sample (Table 5), confirming the results obtained by (Trindade *et al.*, 2008).

All the concentrations with added pequi peel extract (0.5%, 1.0% and 1.5%) maintained sausages with lower rancidity up to 42 days of storage 0.847 ± 0.12, 0.445 ± 0.12 and 0.421 ± 0.10 mg MDA. Kg^{-1} sample, respectively, while the control showed 0.11 ± 1.375 mg MDA. Kg^{-1} sample. Nevertheless, studies show that TBARS values up to 1.59 mg MDA. kg^{-1}

Table 6. Mean values of total aerobic mesophilic and psychrotrophic counts for samples of chicken sausage; control and with different added concentrations of extract of pequi (*Caryocar brasiliense* Camb.) peel during storage at 4ºC (±1ºC)

Total mesophilic aerobic (Log CFU.g^{-1})				
Days of storage	Control	0.5% EPP*	1.0% EPP	1.5% EPP
1	3.04^{b} ± 0.051	3.19^{a} ± 0.001	3.07^{b} ± 0.026	3.24^{a} ± 0.007
7	3.62^{b} ± 0.113	3.90^{a} ± 0.104	3.70^{ab} ± 0.093	3.60^{b} ± 0.118
14	4.64^{b} ± 0.110	4.89^{b} ± 1.039	4.79^{b} ± 0.442	5.99^{a} ± 0.042
21	4.94^{a} ± 0.007	3.11^{b} ± 0.069	4.63^{a} ± 0.087	4.94^{a} ± 0.059
28	6.52^{a} ± 0.062	5.20^{b} ± 0.042	5.51^{b} ± 0.096	5.67^{b} ± 0.247
35	7.14^{a} ± 0.115	6.41^{b} ± 0.066	6.43^{b} ± 0.088	6.28^{b} ± 0.040
Psychrotrophic bacteria (Log CFU.g^{-1})				
Days of storage	Control	0.5% EPP	1.0% EPP	1.5% EPP
1	2.98^{b} ± 0.102	3.27^{ab} ± 0.038	3.48^{a} ± 0.067	3.23^{ab} ± 0.229
7	3.16^{b} ± 0.277	4.54^{a} ± 0.141	3.24^{b} ± 0.080	3.22^{b} ± 0.012
14	3.93^{c} ± 0.517	5.83^{a} ± 0.018	4.72^{b} ± 0.171	5.21^{ab} ± 0.171
21	4.53^{b} ± 0.027	5.78^{a} ± 0.301	4.83^{b} ± 0.056	5.56^{a} ± 0.217
28	6.72^{a} ± 0.136	6.49^{a} ± 0.141	5.90^{b} ± 0.044	5.73^{b} ± 0.114
35	7.76^{a} ± 0.091	7.53^{b} ± 0.032	6.82^{c} ± 0.023	6.59^{d} ± 0.186

Values presented as mean ± standard deviation. Different small letters in the same row indicate significant difference ($p < 0.05$), Tukey's test. *EPP: Extract of pequi peel.

Table 7. Scores assigned by the testers and acceptability index (%) for colour, odour, taste, texture and appearance for samples of chicken sausage; control and different added concentrations of extract of pequi (*Caryocar brasiliense* Camb.) peel on days 6 and 20 of storage at 4°C (± 1ºC)

Sensory characteristics				
After 6 days storage				
Attributes	Control	0.5% EPP*	1.0% EPP	1.5% EPP
Colour	5.10^{ab} ± 1.129	5.44^{a} ± 0.951	5.42^{a} ± 1.263	4.68^{b} ± 1.301
Odour	5.20^{a} ± 1.107	5.20^{a} ± 1.088	5.26^{a} ± 1.175	4.80^{a} ± 1.143
Taste	5.46^{ab} ± 1.100	5.66^{a} ± 1.062	5.62^{a} ± 1.176	4.92^{b} ± 1.243
Texture	5.50^{ab} ± 1.074	5.70^{ab} ± 1.035	5.76^{a} ± 0.981	5.16^{b} ± 1.131
Appearance	5.34^{ab} ± 1.136	5.54^{a} ± 1.147	5.56^{a} ± 1.198	4.80^{b} ± 1.245
After 20 days storage				
Attributes	Control	0.5% EPP	1.0% EPP	1.5% EPP
Colour	5.06^{a} ± 1.252	5.16^{a} ± 1.076	4.86^{a} ± 1.161	4.74^{a} ± 1.337
Odour	5.12^{a} ± 1.206	4.82^{a} ± 1.137	4.84^{a} ± 1.095	4.52^{a} ± 1.233
Taste	5.18^{a} ± 1.380	5.32^{a} ± 1.347	4.96^{a} ± 1.370	4.64^{a} ± 1.352
Texture	4.96^{a} ± 1.355	5.50^{a} ± 1.165	4.96^{a} ± 1.324	5.02^{a} ± 1.116
Appearance	4.96^{a} ± 1.384	5.54^{a} ± 1.061	5.04^{a} ± 1.293	4.66^{a} ± 1.379
Acceptability index				
Attributes	Control	0.5% EPP	1.0% EPP	1.5% EPP
	6 20	6 20	6 20	6 20
Colour	72.86 72.29	77.71 73.71	77.43 69.43	66.86 67.7
Odour	74.29 73.14	74.29 68.86	75.14 69.14	68.57 64.5
Taste	78.00 74.00	80.86 76.00	80.29 70.86	70.29 66.2
Texture	78.57 70.86	81.43 78.57	82.29 70.86	73.71 71.7
Appearance	76.29 70.86	79.14 74.86	79.43 72.00	68.57 66.5

Values presented as mean ± standard deviation. Different letters in the same row indicate significant difference ($p < 0.05$), Tukey's test. Scores: 1 = extremely dislike, 2 = dislike very much, 3 = dislike moderately, 4 = neither liked or disliked; 5 = like moderately, 6 = like very much; 7 = extremely like.
*EPP: Extract of pequi peel.

sample are considered to be too low to be perceived by sensory analysis and do not cause alarm for human health (Torres and Okani, 1997). Thus, it was observed that the pequi peel extract added to fresh sausages acted as an antioxidant.

Microbiological stability

The results for coagulase-positive *Staphylococcus*, *Clostridium sulfite* reducer and coliforms at 45°C were less than 1 log CFU g^{-1} and there was an absence of *Salmonella* spp in 25 g of sample. The National Agency for Sanitary Surveillance (ANVISA), through Resolution RDC No. 12, 2/1/2001 (Brasil, 2001), establishes the technical regulation of microbiological standards for food, and for fresh meat products the maximum values allowed for coagulase-positive *Staphylococcus* and for *Clostridium sulfite* reducer

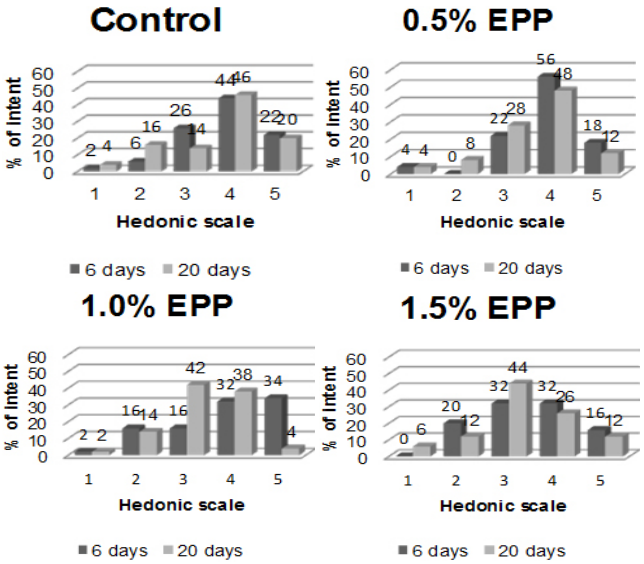

Figure 1. Intention to purchase chicken sausages at 6 and 20 days of storage
1 = I definitely would not buy, 2 = I probably would not buy, 3 = maybe/maybe not, 4 = I would probably buy, 5 = I would definitely buy.

at 46°C is 3 x 10^{3} CFU.g^{-1} for coliforms, at 45°C it is 5 x 10^{3}, and for *Salmonella* spp it is absence in 25 g of sample. Table 6 presents the values found in the microbiological mesophilic and psychrotrophic analyses. It can be seen that the sausages were prepared within the limits prescribed by law, demonstrating that the processing was carried out in adequate conditions of hygiene and in compliance with good manufacturing practices.

To check the shelf life of chicken sausages prepared and stored under refrigeration at 4ºC (± 1°C) counts of aerobic mesophilic and psychrotrophic substances were performed and it was noted that on the twenty-eighth day of storage all treatments with added extract of pequi peel were less than 10^{6} CFU g^{-1}, with the exception of the psychrotrophic substances after 28 days in the lowest concentration of the extract (0.5%). According to Terra (1998), a count of up to 10^{6} CFU g^{-1} is considered to be an acceptable level of microbial contamination in food, which also indicates the sanitary quality of foods (Franco; Landgraf, 1999).

After 28 days of storage, the mesophilic values of the chicken sausages containing added extract were still acceptable; they were lower and significantly different ($p < 0.05$) at all added concentrations, 0.5, 1.0 and 1.5%, compared to the control. At the end of the storage period, 35 days, the count of mesophilic aerobic micro-organisms for all treatments showed figures greater than 10^{6} CFU g^{-1}.

Sensory analysis

The attributes of colour, odour, taste, texture and

appearance, and the acceptability index are presented in Table 7. The grades awarded for the analysed attributes at six days of storage were between 5 and 6, classified as "liked moderately" and "liked a lot" on the seven-point hedonic scale. The control did not differ significantly ($p > 0.05$) from the treatments with added extract regarding the the attribute of odour analysis.

However, the values found after 20 days of storage showed no significant difference ($p > 0.05$) among the treatments, but the average grade of the attributes decreased to 4 and 5, classified as "neither liked or disliked" and "liked moderately" on the scale that was used; the decreasing acceptability of the sausages on the twentieth day was probably a result of the rancid taste. In the acceptability index for the chicken sausages with added extract of pequi peel, the values of all the attributes in the first sensory evaluation were greater than 70% except for colour, odour and appearance of the sausage with the highest concentration of extract (1.5%); consequently, this concentration negatively affected the perception of the tasters. According to Monteiro (1984), an acceptability index is considered to be good when its value is ≥ 70%, so it can be stated that the addition of the extract at a concentration of up to 1.0% did not affect the acceptability of the evaluated attributes.

The results of the intent to purchase test (Figure 1), conducted at six days of storage, showed that the sausage with 1.0% added extract of pequi peel was preferred (34%), equivalent to the term "I would definitely buy" on the scale, followed by the control sausage (22%), the sausage with 0.5% extract of pequi peel (18%) and the sausage with 1.5% extract (16%). Similar results were found by Viera (2012) for Tuscan sausage with 0.5% added extract of propolis, which obtained the highest purchase intent value (34%), equivalent to the term "certainly would buy" on the scale, followed by the control sausage (30%) and the sausage with 1% propolis extract (22%).

Conclusion

The chemical composition of the sausages with added extract of pequi peel and the control were in accordance with the Standard of Identity and Quality. At the end of storage, all the treatments showed a decrease in the L^* values, indicating a darkening of the product. The TBARS values tended to increase over time and the sausages with added extract of pequi peel were free from rancidity up to 42 days, indicating a higher antioxidant capacity due to an increase in the concentration of added extract. By the twenty-eighth day of storage the aerobic mesophilic and psychrotrophic counts for all the treatments with added extract of pequi peel were less than 10^6 $CFU.g^{-1}$, indicating microbiological stability. The results of sensory analysis after six days of storage were superior to those performed after twenty days, indicating a reduction in the acceptability of sausages, probably due to the rancid flavour. The results of the intent to purchase test performed after six days of storage showed that the sausage with 1.0% added extract of pequi peel was preferred.

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