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Effect of extract of chia seed *(Salvia hispanica)* as an antioxidant in fresh pork sausage

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Abstract

This study aimed to verify the effect of chia seed extract *(Salvia hispanica)* at concentrations of 0%, 1%, 1.5% and 2% as an antioxidant in fresh pork sausage and to evaluate the chemical composition, pH, lipid stability, color, microbiological stability and sensory attributes during refrigerated storage. The analysis of moisture, fat, ash, carbohydrates and proteins were determined by AOAC (2005); lipid stability by TBARS values; color by Minolta colorimeter; microbiological analyses as recommended RDC no. 12; and sensory analysis through the affective test. The results regarding chemical composition showed that the sausages were in accordance with the relevant Standard of Identity and Product Quality. The pH of the sausage increased over time in storage. After 28 days of storage, TBARS values were 1.12 mg MDA/kg for the treatment with 2% chia extract and 1.64 mg MDA/kg for the control treatment. With regard to the color of the product, a tendency to browning was observed. Microbiological analysis showed that the sausages were within the limits established by legislation. The sensory characteristics were maintained. The extract of chia seed *(Salvia hispanica)* at a concentration of 2% was shown to inhibit lipid oxidation of the pork sausages and was viable to be used as a natural antioxidant.

Introduction

Lipids are subject to oxidation reactions that compromise their quality. One of the major deteriorative reactions of meat products is lipid oxidation, which limits the shelf life and stability of these commercial foods and is responsible for serious losses in the food industry (Terra, 2008). The products of this reaction are undesirable, not only because of changes in organoleptic characteristics (changes in color of the meat and fat, and the production of offensive odors and flavors), but also because of the destruction of essential constituents, which cause a decrease in the nutritional value of food and the formation of compounds that are toxic to the human body, and which make these products unfit for consumption (Brum, 2009; Yunes, 2010).

The prevention of lipid oxidation is one of the most desired innovations in the meat industry, and an alternative method to reduce lipid oxidation is to include antioxidants in these products (Franco *et al*., 2012). Interest in natural antioxidants has increased recently due to negative consumer perceptions about the safety of synthetic antioxidants, which have been restricted due to their carcinogenic potential, as well as other harmful health effects (Velasco, 2005). Ixtaina *et al*. (2011) report that chia seed *(Salvia hispanica)* contains some compounds with potent antioxidant activity, such as myricetin, quercetin, kaempferol and caffeic acid, which prevent the rancidity of unsaturated fatty acids in foods that contain such acids. However, evaluation of the antioxidant activity of chia seeds used in meat products is not well known, providing, among others, an important reason to incorporate it into pork sausage.

Thus, the purpose of this study was to assess the effect of chia seed extract *(Salvia hispanica)* as an antioxidant in fresh pork sausage and to evaluate the chemical composition, pH, color, TBARS values, microbiological stability and sensory analysis during refrigerated storage at 4°C.

Materials and Methods

Raw material

The pork was donated by the Aurora Alimentos Cooperative, Chapecó, SC-Brazil. The other ingredients used in the formulation of the sausages were purchased from commercial establishments in the municipality of Santa Maria, RS.

Obtaining the extract of chia seeds

The extract was obtained by stirring, according to the methodology described by Caudillo *et al.* (2008),

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with modifications. The extract was prepared from chia seeds that had been previously dried in a forced air oven at 55°C for 24 h. They were then ground in an analytical mill cooled to 4°C (Quimis, model Q 298A21, Brazil) with the aid of ultra thermostatic bath (Solab, model SL-152/10) and then standardized at 60 mesh particle size (0.25 mm), weighed, and 80% hydroethanol solution at a ratio of 1:10 (1 g ground seeds : 10 mL 80% hydroethanol solution) was added. After the addition of the solvent, the mixture was brought to a ultra thermostatic bath at a controlled temperature of 60°C (Solab, model SL-152/10) and subjected to constant stirring using a shaker (Marconi MA- 039) for 60 minutes. Subsequently, the extract was filtered through filter paper and concentrated on a rotary evaporator under vacuum (Fisatom 802) using a temperature of 60°C, for ethanol evaporation. Then the volume was completed with distilled water to keep the initial concentration, and packaged in amber flasks.

Preparation of pork sausage

For the preparation of the pork sausages, the procedures described by Terra (1998) were taken into consideration (Table 1). The pork sausage was prepared from ground meat (Jamar, PJ22 grinder, Jamar Ltda, São Paulo, Brazil) using a 10 mm disk. It was then transported to the mixing machine (Jamar, MJI 35) to receive the remaining ingredients, including pre-defined concentrations of chia seed extract, and mixed to obtain a bind. The treatments were as follows:

Control – without the addition of chia extract (0% CE);
Treatment 1 – addition of 1% chia extract (1% CE);
Treatment 2 – addition of 1.5% chia extract (1.5% CE);
Treatment 3 – addition of 2% chia extract (2% CE).

The meat mixtures were packed into pork intestine casings cleaned with 1% acetic acid and tied into wedges and were then packed in plastic bags and identified. The sausages were stored at +4°C (± 1°C).

Table 1. Formulation of pork sausage

Ingredients	Quantity (%)
Pork meat	92-94
Water/ice	3
Common salt	2.2
Rapid cure	0.25
Ground white pepper	0.1
Minced garlic	0.2
Flavor enhancer	0.05
Sodium erythorbate	0.1
Condiment for Tuscan sausage	0.1
Extract of chia seeds	0-2

Chemical composition of the product

First, the samples were crushed in a multiprocessor to form a smooth paste. The determination of moisture, minerals and proteins were performed according to official methods (AOAC, 2005). Carbohydrates were obtained by difference from the other fractions. Lipids were determined by the butyrometric method, according to Terra and Brum (1988).

Determination of pH

Measurement of pH was performed at 1, 7, 14, 21 and 28 days storage. Ten grams of sample was homogenized with distilled water (1:10 w/v) in a blender. The homogenate was subjected to the electrodes of a Digimed pH meter and readings were performed in triplicate (Terra; Brum, 1988).

Lipid oxidation – TBARS

The assessment of lipid oxidation of the sausages was conducted by the Thiobarbituric Acid Reactive Substances (TBARS) test, according to Raharjo, Sofos and Schmidt (1992). TBARS values were determined in triplicate for each sample after 1, 7, 14, 21 and 28 days of storage and the results were expressed as mg malonaldehyde/kg of sample.

Determintion of color

Coloration was determined using a colorimeter (Minolta Chroma Meter CR-300) and measured at 1, 7, 14, 21 and 28 days storage. The results were expressed as L^*, which is the percentage of light, ranging from black (0%) to white (100%); a^*, where $-a^*$ represents direction toward green and $+a^*$ represents direction toward red; b^*, where $-b^*$ represents direction toward blue and $+b^*$ toward yellow; C^*(saturation index) and h^* (hue angle). For each treatment, the average value of five readings at different points was obtained.

Microbiological analyses

The analyses of coagulase positive *Staphylococcus*, coliform count at 35ºC and 45ºC, *Salmonella* sp and sulphite-reducing *Clostridia* were performed only on the first day of storage (Brazil, 2003). The analyses of total mesophilic and psychrotrophic microorganisms were performed at 1, 7, 14, 21 and 28 days of storage at +4°C (APHA, 2001).

Sensory analysis

This study was approved by the Human Research Ethics Committee (CEP - CONEP) at the Federal University of Santa Maria, UFSM, according to CNS Resolution 196/96 under no. CAAE 16762013.2.0000.5346. For the evaluation of the

Table 2. Chemical composition of fresh pork sausage with different added concentrations of extract of chia seeds *(Salvia hispanica)* stored at 4ºC for 28 days

	Moinsture	Protein	Ash	Fat	Carbohydrate
0% CE	56.58±0.26^{a}	12.97±0.09^{a}	3.28±0.01^{a}	24.85±0.14^{a}	2.32±0.40^{a}
1% CE	56.86±0.22^{a}	12.90±0.11^{a}	3.18±0.08^{ab}	24.81±0.14^{a}	2.25±0.35^{a}
1.5% CE	56.98±0.11^{a}	12.55±0.36^{a}	3.06±0.01^{b}	24.88±0.10^{a}	2.53±0.25^{a}
2% CE	56.69±0.23^{a}	12.80±0.18^{a}	3.22±0.05^{a}	24.88±0.14^{a}	2.41±0.38^{a}

a: Means in the same column with the same superscript letters do not differ significantly by Tukey's test ($p > 0.05$)
Means ± standard deviation of analyses in triplicate.
CE= chia extract

sausages, acceptance testing was used through a hedonic seven-point scale (where 7 represents "highly liked" and 1 corresponds to "disliked extremely") for color, odor, taste and appearance. Regarding purchase intention, the attitude assessment test was used with a five-point scale (where 5 corresponds to "would definitely buy", and 1 corresponds to "would definitely not buy") (Dutcosky, 2011).

Sensory analysis, using 50 untrained tasters, occurred on the 10th day after the preparation of the sausages at the Sensory Analysis Laboratory, Department of Food Science and Technology, Federal University of Santa Maria, UFSM. The samples from each treatment were roasted for 45 minutes at a temperature of 180°C, sliced, coded with 3 random digits, and presented in a monadic way to tasters on disposable dishes.

Statistical analysis

The analyses were conducted in triplicate. The results were submitted 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 analyzed using the Statistica 8.0 (STATSOFT, INC) program.

Results and Discussion

Chemical Composition

The results for the chemical composition of pork sausages are displayed in Table 2 and they show that the products were in accordance with the Standard of Identity and Product Quality (Brasil, 2000), which establishes the maximum value of 70% for moisture, a maximum of 30% fat, 12% minimum for protein and maximum 0.1% of calcium. The legislation for fresh sausage does not define a standard for ash content.

pH

The results of the effect of chia seed extract *(Salvia hispanica)* on the pH of the fresh pork sausages stored at 4°C for 28 days are shown in Table 3. Regarding the storage time, it was possible to observe a tendency for pH values to increase in all of the studied treatments. Similar results were reported by Brannam (2008), where there was an increase in pH in refrigerated chicken and by Georgantelis *et al*. (2007) in a study of pork sausage refrigerated for 20 days. This fact is attributed to a rise in the counts of psychrotrophic microorganisms, which produce protease. When protease production is started by bacteria, the latter use amino acids as growth substrate, instead of using glucose. The use of amino acids leads to increased pH due to the formation of ammonia and amines (Terra; Brum, 1988).

The pH values were not significantly different ($p > 0.05$) between the treatments during the 28 days of storage. Similar results were found by Brum (2009), who describes the natural antioxidant effect of macela *(Achyrocline satureioides)* and yerba mate *(Ilex paraguariensis)* in preparing Tuscan sausage, where the pH values showed no significant difference ($p > 0.05$) between all the analyzed treatments.

Lipid-oxidation TBARS

As can be seen in Table 3, after 28 days of storage there was a significant increase in TBARS values for all the analyzed treatments, evidencing the occurrence of lipid oxidation. It can also be noted that between 1 and 7 days of storage, the values of TBARS increased in proportion to increases in the concentration of chia extract in the treatments. In contrast to its *in vitro* antioxidant properties, studies suggest that some polyphenolic compounds have pro-oxidant properties (Murzakhmetora *et al*., 2008). However, the margin between the quantity that is functionally required for optimal antioxidant performance and a pro-oxidant dose may be small, and this fact should be considered when these ingredients are used to enrich food (Rietjens *et al*., 2002). Future research using the chia seed extract in more complex meat

Table 3. Values of pH and TBARS (mg MDA/kg sample) for fresh pork sausages with different added concentrations of extract of chia seeds *(Salvia hispanica)* stored at 4°C for 28 days

	pH				
	Day 1	Day 7	Day 14	Day 21	Day 28
0%CE	5.78 ± 0.37^{aA}	6.15 ± 0.25^{aA}	6.31 ± 0.18^{aA}	6.39 ± 0.19^{aA}	6.24 ± 0.37^{aA}
1%CE	6.15 ± 0.01^{cA}	6.36 ± 0.07^{abA}	6.45 ± 0.04^{abA}	6.36 ± 0.11^{bA}	6.54 ± 0.04^{aA}
1.5%CE	6.13 ± 0.08^{bA}	6.28 ± 0.03^{bA}	6.35 ± 0.17^{abA}	6.37 ± 0.06^{abA}	6.60 ± 0.06^{aA}
2% CE	6.21 ± 0.09^{bA}	6.49 ± 0.10^{aA}	6.47 ± 0.12^{aA}	6.18 ± 0.01^{bA}	6.59 ± 0.04^{aA}
	TBARS				
	Day 1	Day 7	Day 14	Day 21	Day 28
0%CE	0.21 ± 0.06^{cD}	0.44 ± 0.02^{cC}	1.16 ± 0.02^{bA}	1.50 ± 0.09^{aA}	1.64 ± 0.10^{aA}
1%CE	0.34 ± 0.01^{cC}	0.38 ± 0.01^{cC}	1.12 ± 0.12^{bA}	1.33 ± 0.11^{abA}	1.54 ± 0.16^{aA}
1.5%CE	0.46 ± 0.03^{cB}	0.54 ± 0.05^{cB}	1.11 ± 0.15^{bA}	1.20 ± 0.01^{abAB}	1.40 ± 0.02^{aA}
2% CE	0.61 ± 0.02^{bA}	0.64 ± 0.02^{bA}	1.05 ± 0.02^{aA}	1.07 ± 0.04^{aB}	1.12 ± 0.03^{aB}

a: Means in the same row with the same superscript letters do not differ significantly by Tukey's test ($p > 0.05$).
A: Means in the same column with the same superscript letters do not differ significantly by Tukey's test ($p > 0.05$).
Means ± standard deviation of analyses in triplicate.
CE= chia extract.

systems is required.

From the 14th until the 28th day of storage, all the treatments with added chia seed extract showed lower TBARS values than the control treatment. However, there was only significant difference ($p < 0.05$) compared to control at 21 and 28 days of storage, when the treatment with the addition of 2% extract of chia showed statistically lower TBARS values.

All the concentrations with added chia seed extract were able to maintain the product at lower oxidation level up to 28 days of storage than the control, which showed 1.64 mg MDA/kg sample. In addition, studies show that TBARS values up to 1.59 mg MDA/kg sample are considered to be too low to be perceived by sensory analysis and do not represent a threat to human health (Torres; Okani, 1997), which makes these sausages suitable for consumption up to 28 days of storage. The samples with chia seed extract showed TBARS values from 1.12 to 1.54 mg MDA/kg.

Bussata (2010) analyzed the effect of the addition of extract of oregano and marjoram in Tuscan sausage and found values of 0.806 mg MDA/kg at the end of 35 days of storage, values that were lower than those found in this study. It is worth noting that formulated products are highly vulnerable to develop lipid oxidation, due to processing conditions, packaging, and storage, and also due to the large fat content. The presence of phenolic compounds and *in vitro* antioxidant activity in chia seeds *(Salvia hispanica)*, has been studied and proven (Sargi *et al*., 2013, Caudillo *et al.*, 2008); however, it has not been as effective as other extracts when applied to pork sausages.

Color

The results obtained for luminosity *(L*)*, redness *(a*)*, yellow *(b*)*, saturation index *(C*)* and hue angle *(h*)* of the pork sausage during storage are presented in Table 4. Regarding the length of storage, all the treatments showed a reduction in the *L** value compared to baseline, indicating the darkening of the product, given that the values of *L** (lightness) range from black (0%) to white (100%) (Ramos; Gomide, 2007). Similar effects of a decreased *L** value were recorded by Biswas; Chatli and Sahoo (2012) for pork stored under refrigeration with the addition of extracts of curry leaves and mint.

Regarding the a^* parameter (where $-a^*$ represents direction toward green and $+a^*$ represents direction toward red), there was significant difference ($p < 0.05$) between the control and the other treatments; the control showed higher results, except on the first day of storage (treatment 1% CE), 7th day of storage (treatment 1.5% CE), 14th day of storage (2% CE treatment) and 21st day of storage (treatment 2% CE), where they were significantly equal to the control ($p > 0.05$).

There was also an increase in the values found at the end of storage as compared to the start, with high values for the a^* parameter related to the concentration of myoglobin and the formation of nitrosomyoglobin during the curing process (Perez-Alvarez *et al*., 1998). It was noted that on the first day of storage, the sausages had the lowest a^* values and there was a significant increase on the 7th day of

Table 4. Parameters of instrumental color (L^*, a^*, b^*, C^* and h^*) from fresh pork sausage with different added concentrations of extract of chia seeds *(Salvia hispanica)* stored at 4°C for 28 days

	Day 1	Day 7	Day 14	Day 21	Day 28
			L*		
0% CE	62.69 ± 0.55^{aA}	60.23 ± 0.91^{aA}	61.76 ± 0.33^{aA}	61.66 ± 0.70^{bA}	59.37 ± 0.36^{aBC}
1% CE	61.95 ± 0.40^{bA}	61.02 ± 0.69^{abA}	61.97 ± 0.87^{aA}	61.05 ± 0.79^{abA}	62.20 ± 0.80^{aA}
1.5% CE	62.71 ± 0.31^{aA}	61.52 ± 0.74^{aA}	62.06 ± 1.04^{aA}	61.29 ± 0.42^{aA}	60.78 ± 0.97^{aAB}
2% CE	62.22 ± 0.35^{aA}	60.19 ± 0.43^{bA}	61.33 ± 0.29^{bA}	61.02 ± 0.60^{bcA}	57.55 ± 0.87^{cC}
			a*		
0% CE	8.83 ± 0.14^{cA}	17.22 ± 0.90^{bA}	18.69 ± 0.17^{aA}	19.35 ± 0.52^{aA}	20.00 ± 0.23^{aA}
1% CE	8.48 ± 0.25^{dAB}	13.11 ± 0.47^{cB}	14.44 ± 1.03^{bcC}	17.23 ± 0.59^{aB}	16.15 ± 0.63^{abB}
1.5% CE	7.61 ± 0.47^{cBC}	18.22 ± 0.92^{aA}	15.33 ± 0.56^{bBC}	17.06 ± 0.52^{abB}	15.54 ± 0.75^{bB}
2% CE	7.34 ± 0.43^{dC}	14.37 ± 0.12^{cB}	16.77 ± 0.88^{bAB}	20.34 ± 0.54^{aA}	16.35 ± 0.81^{bB}
			b*		
0% CE	12.29 ± 0.76^{aB}	10.98 ± 0.39^{abA}	10.85 ± 0.36^{bA}	11.97 ± 0.31^{abA}	11.97 ± 0.66^{abA}
1% CE	13.66 ± 0.35^{aA}	9.97 ± 0.83^{cA}	10.93 ± 0.48^{bcA}	11.68 ± 0.08^{bA}	11.48 ± 0.19^{bA}
1.5% CE	12.19 ± 0.17^{aB}	10.78 ± 0.60^{bA}	10.73 ± 0.45^{bA}	11.81 ± 0.10^{abA}	11.39 ± 0.54^{abA}
2% CE	12.20 ± 0.25^{aB}	10.19 ± 0.36^{cA}	10.82 ± 0.23^{bcA}	12.51 ± 0.54^{aA}	11.14 ± 0.17^{bA}
			C*		
0% CE	15.13 ± 0.53^{dAB}	20.43 ± 0.82^{cA}	21.60 ± 0.43^{bcA}	22.53 ± 0.60^{abAB}	23.38 ± 0.54^{aA}
1% CE	16.07 ± 0.66^{cA}	17.72 ± 0.70^{bcB}	18.12 ± 0.95^{bB}	20.96 ± 0.73^{aBC}	19.52 ± 0.70^{abB}
1.5% CE	14.37 ± 0.25^{cB}	20.03 ± 0.42^{abA}	18.47 ± 0.29^{bB}	20.75 ± 0.60^{aC}	18.84 ± 0.85^{abB}
2% CE	14.26 ± 0.31^{aB}	17.78 ± 0.74^{dB}	21.39 ± 0.98^{bA}	23.78 ± 0.42^{aA}	19.64 ± 0.97^{cB}
			h*		
0% CE	55.60 ± 0.26^{aB}	32.26 ± 0.87^{bC}	30.06 ± 0.65^{cC}	32.00 ± 0.72^{bB}	31.90 ± 0.52^{bC}
1% CE	58.16 ± 0.66^{aA}	36.80 ± 0.70^{bcA}	37.76 ± 0.95^{bA}	34.16 ± 0.73^{dA}	34.93 ± 0.70^{cdA}
1.5% CE	58.46 ± 0.66^{aA}	30.50 ± 0.20^{dD}	35.13 ± 0.51^{bB}	34.66 ± 0.85^{bcA}	33.46 ± 0.40^{cB}
2% CE	59.56 ± 0.85^{aA}	34.96 ± 0.70^{bB}	30.33 ± 0.15^{cC}	30.76 ± 0.61^{cB}	33.66 ± 0.51^{bAB}

a: Means in the same row with the same superscript letters do not differ significantly by Tukey's test ($p > 0.05$).
A: Means in the same column with the same superscript letters do not differ significantly by Tukey's test ($p > 0.05$).
Means ± standard deviation of analyses in quintuplicate.
CE= chia extract

storage. This was because the meat being used was at low temperatures at the time of preparation of the sausages, which delayed the curing reaction, which occurred only after the first day of storage.

The b^* parameter (where $-b^*$ represents direction toward blue and $+b^*$ represents direction to yellow) decreased over storage time; this was contrary to the expected behaviour because the treatments underwent oxidation during storage, and therefore should have presented an increase in b^* values. This fact is probably related to the increase in the values of the a* parameter of the treatments, which masked the yellowing of the sausages due to lipid oxidation

The degree of saturation (C^*) and hue angle (h^*) are measurements derived from a^* and b^*. The chromacity or chroma (C^*) expresses the intensity of color, i.e. the saturation in terms of the pigments of this color. Chroma values near zero represent neutral colors (grays), while values close to 60 express vivid colors (Mendonça *et al*., 2003). Table 4 shows that the C^* values increased during storage, with more vivid coloring. The hue angle (h^*) is the quantity associated with the wavelengths of the visible spectrum, representing the quality of the color (blue, red, yellow, etc.) and allowing differentation (Ramos; Gomide, 2007). Table 4 shows that on the 1st, 7th and 28th days of storage there was significant difference ($p < 0.05$) compared to the control and the other treatments, where the control treatment showed higher results than the other treatments. Regarding the length of storage, all the treatments showed reduced h^* values.

Microbiological Analysis

According to the RDC no. 12 resolution of January 2, 2001 (Brasil, 2001), which approved the Technical Regulation on Microbiological Standards for Foods, the tolerance in fresh pork sausage for coagulase positive Staphylococcus and sulphite-reducing *Clostridia* to 46°C is 3 x 10^3 CFU/g (3.47 Log CFU/g); for coliforms at 45°C it is 5 x 10^3 CFU/g (3.69 Log CFU/g) ; and for *Salmonella* spp it is absence in 25 g . The results for coagulase positive *Staphylococcus*, sulphite-reducing *Clostridia* and coliforms at 45°C found in this study showed lower results than 1 Log CFU/g and *Salmonella* spp was absent in 25 grams of sample (data not shown). Thus, the prepared pork sausages were within the limits allowed by law, demonstrating that processing was carried out in adequate conditions of hygiene and in compliance with good manufacturing practices.

Table 5. Count of total aerobic mesophilic and psychrotrophic microorganisms (Log CFU/g) in samples of fresh pork sausage with different added concentrations of extract of chia seed *(Salvia hispanica)* stored at 4°C for 28 days

Mesophilic					
	Day 1	Day 7	Day 14	Day 21	Day 28
0%CE	3.36 ± 0.05^{dA}	5.69 ± 0.04^{cA}	6.57 ± 0.07^{bA}	6.53 ± 0.20^{bAB}	7.81 ± 0.01^{aB}
1%CE	2.94 ± 0.13^{cA}	4.79 ± 0.03^{bAB}	6.47 ± 0.21^{aA}	6.57 ± 0.01^{aA}	6.89 ± 0.08^{aC}
1.5%CE	3.27 ± 0.06^{cA}	3.79 ± 0.23^{cB}	6.27 ± 0.03^{bA}	5.96 ± 0.02^{bB}	7.41 ± 0.18^{aB}
2% CE	2.97 ± 0.19^{dA}	4.53 ± 0.45^{cB}	6.20 ± 0.42^{bA}	6.97 ± 0.20^{bA}	8.89 ± 0.01^{aA}
Psychrotrophic					
	Day 1	Day 7	Day 14	Day 21	Day 28
0%CE	2.83 ± 0.04^{dA}	5.58 ± 0.15^{cB}	6.58 ± 0.17^{bA}	6.89 ± 0.02^{abA}	7.28 ± 0.01^{aB}
1%CE	2.72 ± 0.15^{dA}	5.51 ± 0.01^{cB}	5.87 ± 0.05^{bBC}	6.52 ± 0.06^{aBC}	6.59 ± 0.04^{aD}
1.5%CE	2.81 ± 0.04^{eA}	5.09 ± 0.05^{dC}	5.84 ± 0.12^{cC}	6.34 ± 0.06^{bC}	6.99 ± 0.01^{aC}
2% CE	2.65 ± 0.02^{eA}	5.96 ± 0.01^{dA}	6.32 ± 0.01^{cAB}	6.65 ± 0.04^{bB}	7.72 ± 0.02^{aA}

a: Means in the same row with the same superscript letters do not differ significantly by Tukey's test ($p > 0.05$).
A: Means in the same column with the same superscript letters do not differ significantly by Tukey's test ($p > 0.05$).
Means ± standard deviation of analyses in quadruplicate.
CE= chia extract.

In order to verify the shelf life of the pork sausages stored under refrigeration, counts of total aerobic mesophilic and psychrotrophic microorganisms were performed (Table 5), which can be used to indicate the health quality and safety of meat products by providing a general estimate of the population of microorganisms present in a wide temperature range; high levels of contamination are associated with low quality and an accelerated process of deterioration (Jay, 2005). According to Terra (1998), a count of up to 10^6 CFU /g (6 Log CFU /g) is considered to be an acceptable range of microbial contamination in foods, which also indicates the sanitary quality of foods.

Table 5 shows that that the count of aerobic mesophilic and psychrotrophic microorganisms gradually increased in all treatments throughout the storage period. The results show that the microbial population of aerobic mesophiles in the pork sausages showed no significant differences ($p > 0.05$) with the addition of chia seed extract on the 1st and 14th of storage. At 21 and 28 days of storage, the treatment with the highest concentration of added extract (2.0% CE) presented statistically greater values ($p < 0.05$) than the other treatments, thus demonstrating that it did not have an antimicrobial effect on the studied microorganisms.

Likewise, the count of psychrotrophic microorganisms (Table 5) varied between all the treatments over all the period of storage; it was not possible to statistically lower values ($p > 0.05$) in the treatments with added chia extract, compared to control. On the 7th and 28th days of storage, the treatment with the highest concentration of chia seed extract (2% CE) had a count that was statistically higher ($p < 0.05$) than the other treatments, highlighting the fact that the chia seed extract did not demonstrate antimicrobial activity. From the 14th day of storage, the treatments had counts of aerobic, mesophilic and psychrotrophic microorganisms above 6.0 Log CFU/g (excluding treatments 1% and 1.5% CE for psychrotrophic microorganisms), which exceededg the acceptable range limit for bacterial contamination (Terra, 1998).

Sensory analysis

The average values assigned to all of the evaluated attributes (Table 6) were around 5, classified as "moderately like" on the seven-point hedonic scale. The attributes of appearance, color and odor showed no significant difference ($p > 0.05$) between the treatments, indicating that there was no interference from the addition of different concentrations of chia seed extract in the pork sausages. As for attribute of taste, there was statistical difference ($p < 0.05$) between the treatments. The treatment with 2.0% added chia extract showed a lower average for the tasters, with a lower acceptability; the addition of natural substances can change the characteristic flavor of these products. However, the treatment with

Table 6. Averages of the scores assigned (using a 7-point hedonic scale) and purchase intent (using a 5-point hedonic scale) for fresh pork sausages with different added concentrations of extract of chia seed *(Salvia hispanica)* stored at 4°C

	Appearance	Color	Odor	Taste	Intention to purchase
0% CE	5.36±0.99^{a}	5.36±1.02^{a}	5.39±0.88^{a}	5.52±0.99^{ab}	3.94±0.99^{a}
1% CE	5.42±1.05^{a}	5.28±1.18^{a}	5.23±1.12^{a}	5.39±1.32^{ab}	3.71±0.76^{a}
1.5% CE	4.78±1.01^{a}	5.32±1.29^{a}	5.44±1.08^{a}	5.89±0.83^{a}	3.92±1.09^{a}
2% CE	5.15±1.28^{a}	5.25±1.13^{a}	5.05±1.22^{a}	5.10±1.31^{b}	3.71±0.74^{a}

a: Means in the same column with the same superscript letters do not differ significantly by Tukey's test ($p > 0.05$). Means ± standard deviation of analyses of 50 tasters.
CE= chia extract.
Scores 7-point hedonic scale.: 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = indifferent; 5 = like moderately; 6 = really like; 7 = like extremely.
Scores 5-point hedonic scale: 1= definitely would not buy; 2 = probably would not buy; 3 = possibly would buy; 4 = probably would buy; 5 = definitely would buy

1.5% added chia extract received the best average values for flavor.

In this context, one of the major concerns when developing a product is to ascertain the intention of purchase by the consumer (Santana *et al*., 2006). The results of the intention to purchase test performed on the 10th day of storage of the sausages are presented in Table 6. The average purchase intention for the sausages was equivalent to the term "would possibly buy" on the scale. There was no statistical difference ($p > 0.05$) between the control treatment and the treatments with added chia extract. This demonstrates that the addition of extract of chia seed had no effect on consumer behavior towards buying the product.

Conclusion

The chemical composition of the sausages with added chia seed extract and the control were in accordance with the Standard of Identity and Product Quality. The mean values of pH in the different analyzed treatments increased during the period of storage. At the end of storage, all the treatments showed a decrease in L^* values, indicating darkening of the product. TBARS levels tended to increase over time in storage. The extract of chia seeds *(Salvia hispanica)* was efficient regarding the oxidative stability of the pork sausage. This was observed from 14 days of storage until the end of the analysis, when all treatments with added chia extract had lower oxidation than the control treatment; the treatment with 2% added chia extract had the lowest lipid oxidation. The microbiological analysis was within the tolerance limits established by Brazilian Legislation for all the treatments. In the sensory analysis, the mean values of the scores of the analyzed attributes were "moderately liked." The test of intention to purchase showed that there was no significant difference in intent to purchase between the control sausage and the sausage with added extract of chia seed. In general terms, the pork sausages containing 2% chia seed extract showed the best results compared to the other concentrations with regard to lipid oxidation.

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