Original

The influence of *achyrocline satureioides* ("Marcela") extract on the lipid oxidation of salami

Influência do extrato de marcela (achyrocline satureioides) na oxidação lipídica de salames

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Abstract

The effect of two levels (0.5 and 1%) of hydroalcoholic extract of *Achyrocline satureioides* on the safety (TBARS values) and quality (pH, water activity, colour, weight loss, and sensorial attributes) of salami was evaluated. The addition of *Achyrocline satureioides* extract decreased TBARS values significantly during the storage of salami when compared to the control, which was elaborated without *Achyrocline satureioides* extract. The treatment with 1% of "Marcela" extract showed larger lipid stability than that of the lot with 0.5%, However, it presented a decrease ($p < 0.05$) in the sensorial acceptance. The two levels of "Marcela" extract did not influence pH, water activity, colour, and weight loss significantly. This study indicates that the hydroalcoholic extract of "Marcela" was effective in decreasing the lipid oxidation and at 0.5% it did not alter the sensorial features; therefore, it may be used in salami to provide safer products for the consumers.

Keywords: *salami; lipid oxidation; natural antioxidant; "Marcela".*

Resumo

O efeito de dois níveis (0,5% e 1%) de extrato hidroalcoólico de marcela (*Achyrocline satureioides*) na segurança (valores de TBARS) e qualidade (pH, atividade de água, cor, perda de peso e atributos sensoriais) de salames foi avaliado. A adição de extrato de marcela diminuiu significativamente os valores de TBARS durante o armazenamento dos salames, comparado ao controle, elaborado sem extrato de marcela. O tratamento com 1% de extrato de marcela mostrou uma maior estabilidade lipídica que o lote com 0,5%, porém apresentou uma diminuição ($p < 0,05$) na aceitação sensorial. Os dois níveis de extrato de marcela não influenciaram significativamente os parâmetros de pH, atividade de água, cor e perda de peso. Este estudo indicou que o extrato hidroalcoólico de marcela foi efetivo na diminuição da oxidação lipídica e que a concentração de 0,5% não alterou as características sensoriais, podendo, portanto, ser utilizada em salames para proporcionar produtos mais seguros aos consumidores.

Palavras-chave: *salame; oxidação lipídica; antioxidante natural; marcela.*

1 Introduction

The shelf life of fermented meat sausages is not usually limited by microbial deterioration, but by chemical or physical alterations. Due to the high percentage of fat and low water activity, lipid oxidation is considered one of the main limiting shelf-life factors of those products.

Lipid oxidation causes modifications of organoleptic features of meat products, such as, for instance, alterations of meat colour and fat, development of unpleasant taste, and smell and decrease in the nutritional value of the product due to the decrease in the content of polyunsaturated fatty acids, whose beneficial effect on consumers' health is well documented (ALEXANDER, 1998; ROSE; CONNOLLY, 1999; BERRA; MONTORFANO; RIZZO, 2005). In addition, some intermediate and final products of oxidation reactions are potentially toxic to human health, such as the oxidation compounds of cholesterol (KUBOW, 1990; PANIANGVAIT et al., 1995) and the polymerization of triglycerides (ALEXANDER, 1978; CHANG; PETERSON; HO, 1978) besides the aldehydes with α and β instaurations including the malonaldehyde, which is known for its toxic, mutagenic and carcinogenic effects (NEWBURG; CONCON, 1980).

The antioxidants present themselves as an alternative to prevent the oxidative deterioration of foods and minimize the oxidative damages in humans. Since the application of synthetic antioxidant in food industry has been target of queries as for its innocuousness, scientific studies have been focused on the search for natural compounds which present this functional property (MELO; GUERRA, 2002).

Extensive research has been focused on discovering new sources of natural antioxidants to be used in meat and meat products (CHEAH; HASIM, 2000; MCCARTHY et al., 2001; KARPINSKA; BOROWSKI; DANOWSKA-OZIEWICZ, 2001; KANATT; CHANDER; SHARMA, 2004; KANATT et al., 2005; CAMPOS et al., 2007). *Achyrocline satureioides*, commonly known as "Marcela", is a medicinal plant used in

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Argentina, Uruguay, Paraguay, and Brazil for its antispasmodic, hepatoprotecting, and coleretic properties. The high content of polyphenolic compounds, most of them flavonoids, and different phenolic acids such as capheic, chlorogenic, and isochlorogenic (SIMÕES et al., 1988; FERRARO; NORBEDO; COUSSIO, 1981) suggests that this plant may have powerful antioxidant effects. There are some reports on the antioxidant properties of "Marcela" (GUGLIUCCI; MENINI, 2002; DESMARCHELIER; COUSSIO; CICCIA, 1998), but its application in meat products has not been studied.

This objective of this study was to evaluate the effect of "Marcela" extract (*Achyrocline satureioides*) on the oxidative stability and on the sensorial characteristics of fermented salami sausages during storage.

2 Material and methods

2.1 Extract preparation

The dry vegetal product (30 g of inflorescence of "Marcela") was homogenized with solvent, transferred to a beaker, and left for 1 hour at room temperature. Next, it was filtrated through filter paper (Whatman number 6). The residue was submitted to two more successive extractions with the objective of fully extract the active principle of the raw material. The 3 aliquots filtered were collected and concentrated by rotary evaporation (Rotavapor® RE 120 - Büchi, Flawil, Switzerland) until 7% of the initial volume, to obtain the crude extract which was kept under refrigeration in glass flask protected from light. In the elaboration of the extract, the liquid-solid ratio was 12:1. The solvent applied in the first extraction was a mixture of ethanol 95% with distilled water (12:1), and in the two following extractions it was ethanol 95%.

2.2 Elaboration of salami

The salami samples were elaborated according to the following formulation: pork (600 $g.kg^{-1}$) and beef (300 $g.kg^{-1}$), lard (100 $g.kg^{-1}$), sodium chloride (25 $g.kg^{-1}$), glucose (5 $g.kg^{-1}$), saccharose (5 $g.kg^{-1}$), commercial mixture of cure, containing nitrate and sodium nitrite (3 $g.kg^{-1}$), sodium ascorbate (2.5 $g.kg^{-1}$), white pepper (2 $g.kg^{-1}$), garlic (3 $g.kg^{-1}$), nutmeg (0.02 $g.kg^{-1}$), and commercial *starter* culture Floracarn SPX (Chr. Hansen) (0.25 $g.kg^{-1}$). Portions of pork meat and beef were ground in disks of 12 and 8 mm, respectively. Pieces of pork fat, lard, were cut in cubes of nearly 1 cm^3 using a knife, After being ground, the mass of meat and the lard were added with sodium chloride and mixed in a blender for 3 minutes for the extraction of myofibrillar proteins. After adding all other ingredients the *starter* culture, previously diluted in chlorine-free distilled water (200 mL of water for each 100 kg of mass of meat), was finally added.

The mass of meat was divided equally into three lots, originating the following treatments: 1) control, without the addition of hydroalcoholic extract of "Marcela"; 2) addition of 0.5% of hydroalcoholic extract of "Marcela"; and 3) addition of 1% of hydroalcoholic extract of "Marcela".

The treatments were stuffed in collagen casings of 60 mm diameter and cut in slices of approximately 15 cm in length. After being stuffed, the samples were subjected to a bath in a 20% solution of potassium sorbate and ripened in a laboratory ripening cabinet (Menoncin, Erechim, Brazil) until reach water activity of 0.87.

The programming of temperature and relative humidity (T°/R.U.%) was the following: first day, temperature 25 °C/R.U. 95%; second day, 24 °C/93%, third day, 23 °C/90%, fourth day, 22 °C/85%, fifth day, 21 °C/80%, sixth day, 20 °C/75%, and from seventh day on, 18 °C/75%. When ready, the guts were removed, and the pieces of the fermented sausages were vacuum-packed and kept at room temperature for 90 days.

2.3 Physicochemical analysis

pH determination

The measurement of pH was performed by homogenizing ten grams of sample with distilled water (1:10). The pH of the homogenized sample was determined by a pH meter (Digimed electrode) (TERRA; BRUM, 1988). The pH determination was performed on days 0, 1, 2, 3, 4, 5, 6, 7, 14 and 19 of manufacturing.

Water activity determination (Aw)

The determination of the Aw was performed by using the gadget Testo 400 CE, (TESTO GMBH & CO.), and were made on days 0, 3, 7, 14, 16 and 19 of manufacturing.

Weight loss

The weight loss was determined by the weight difference among ten sausages per batch just after the stuffing process and after the end of sausage production (TERRA; BRUM, 1988).

Colour measurement

The colour determination was performed with a Minolta Chroma Meter CR-300 equipment (MINOLTA). The results were expressed as L* (lightness), a* (red index), and b* (yellow index). The determinations were performed on days 0, 3, 7, 14 and 19 of manufacturing.

Evaluation of the lipidic oxidation

Thiobarbituric Acid Reactive Substances (TBARS) resulting from lipidic oxidation were determined according to Raharjo and Sofos (1993) method. The TBARS values were determined in triplicate for each sample after zero, 15, 30, 45, 60, 75, and 90 days of storage, and the results were expressed in mg of malonaldehyde for kg of sample.

2.4 Sensorial analysis

The global acceptance of the products was evaluated at zero 30, 60, and 90 days of storage with the aid of a panel constituted by 30 non-trained tasters, consumers of salami, using a structured hedonic scale of seven points varying from "I disliked it very much" (score 1) to "I liked it very much" (score 7). The samples were offered to the tasters in white plastic plates, codified with three digits, followed by a glass of water and cream crackers (MORAES, 1988).

2.5 Statistical analysis

All determinations were made in triplicate and the data were evaluated through the variance analysis (ANOVA). Means were compared by the Tukey test, considering the significance level of de 5% ($p < 0.05$) using the statistical pack SAS (1996), version 6.12.

3 Results and discussion

3.1 Physicochemical analysis

The changes in the pH values during the manufacturing period of the salami are presented in Figure 1. During the first seven manufacturing days, a decrease in the pH values from 5.80 to nearly 4.95 was observed, probably due to the accumulation of lactic acid formed by the lactic acid bacteria (TERRA, 1998). The decline in the pH value during the first days of fermentation is very important for the production of high quality safe salami due to the inhibition of undesirable microorganisms, conversion and stabilization of colour, and formation of desireable taste and smell compounds (LÜCKE, 1994). After the seventh day, the pH values increased up to 5.14. This may be attributed to the production of ammonia and biogenic aminates as a result of the enzymatic activity (LÜCKE, 1998; BOZKURT; ERKMEN, 2002). The "Marcela" extract did not affect significantly ($p > 0.05$) the pH values during the period of salami production, suggesting that its addition does not cause alterations in the fermentative process.

The changes in water activity (Aw) during the period of salami production are shown in Figure 2. Aw decreased during the production reaching the value of 0.87 in all treatments after 19 days. This reduction is due to the decrease in the pH because the water retention capacity of meat proteins decreases when the pH gets closer to its isoelectric point accelerating the dehydration and consequently reducing the Aw (CHASCO; LIZASO; BERIAIN, 1996). The statistical analysis showed that the addition of "Marcela" extract did not influence significantly ($p > 0.05$) the water activity during the production period.

The weight loss was 46.92, 46.78, and 45.86% in the control lot and in treatments with 0.5 and 1% of "Marcela" extract, respectively. No significant difference ($p > 0.05$) was observed among the treatments. Those values are very close to the range of 30 to 40%, which is considered ideal for dry fermented products (RUST, 1994).

Table 1 presents the colour parameters during the salami production. The L* values decreased in all treatments during the 19 days of production, and no significant difference was observed among the treatments. This decrease represents the formation of a dark colour due to the darkening reactions (BOZKURT; BAYRAM, 2006). Similarly, Kayaardi and Gök (2003) verified that the L* values in salami usually decrease during the maturation period.

The a* values increased during the whole production period (Table 1). During the first days of fermentation, the nitric oxide that is already present in the meat combines with myoglobin producing tnitrosomyoglobin (LÜCKE, 1994). Since this pigment has red colour, the a* values increased during the salami elaboration. The addition of "Marcela" extract reduced significantly the a* values at the beginning (0 day) and on the 3rd day of production. However, from the 7th day on, although lower in the treatments with "Marcela" extract, the a* values did not differ significantly from the control.

The b* values decreased in all treatments during the salami production, and no significant difference was observed among them (Table 1). Those results agree with the data obtained by Perez-Alvarez et al. (1999), who observed a decrease in the b* values of salami during fermentation and maturation. The authors attributed this decrease to the consumption of oxygen by microorganisms and to the consequent decrease in oxymyoglobine, which contributed to the yellow colouring

The TBARS test is the most usual method to follow the evolution of the lipidic oxidation in meat and meat products (RAHARJO; SOFOS, 1993). The changes in the TBARS values

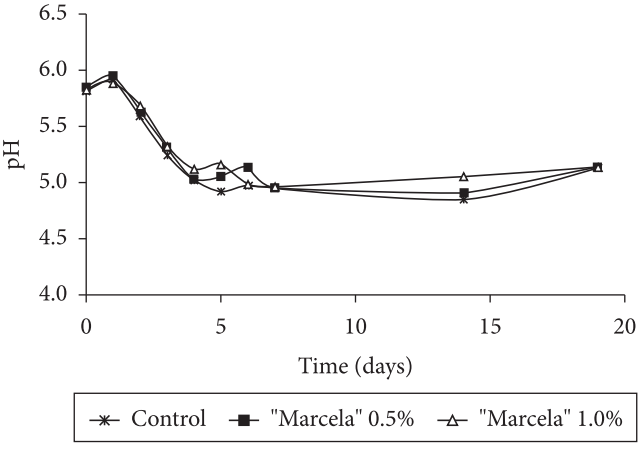

Figure 1. Evolution of pH during the production period of the salami formulated with different levels of hydro-alcoholic extract of "Marcela". Control: without addition of "Marcela" extract; "Marcela" 0.5%: addition of 0.5% of hydro-alcoholic extract of "Marcela"; "Marcela" 1%: addition of 1% of hydro-alcoholic extract of "Marcela".

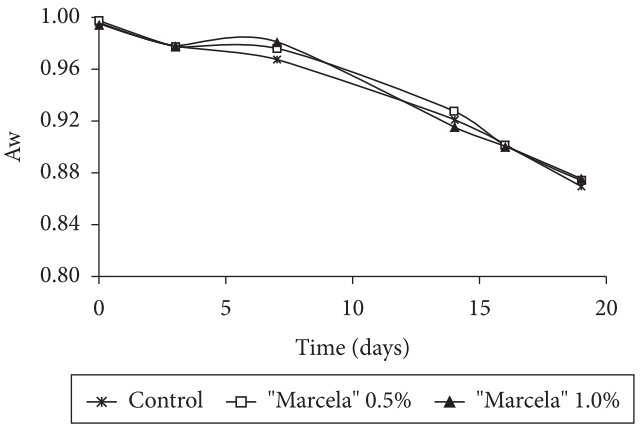

Figure 2. Evolution of water activity (Aw) during the production period of the salami formulated with different levels of hydro-alcoholic extract of "Marcela". Control: without addition of "Marcela" extract; "Marcela" 0.5%: addition of 0.5% of hydro-alcoholic extract of "Marcela"; "Marcela" 1%: addition of 1% of hydro-alcoholic extract of "Marcela".

Table 1. Mean values (± standard deviation) of colour parameters in salami formulated with different levels of hydroalcoholic extract of "Marcela"

Days	Treatments*	L*	a*	b*
0	Control	51.18a ± 3,48	14.90a ± 0,95	12.06a ± 0,57
	"Marcela" 0.5%	54.39a ± 0,18	11.23b ± 0,15	12.89a ± 0,21
	"Marcela" 1%	53.39a ± 1,80	11.77b ± 0,72	13.23a ± 0,14
3	Control	50.01a ± 0,89	21.15a ± 0,13	11.56a ± 0,28
	"Marcela" 0.5%	49.16a ± 1,11	18.11b ± 0,29	11.11a ± 0,26
	"Marcela" 1%	49.26a ± 0,67	17.13b ± 0,43	11.39a ± 0,20
7	Control	48.86a ± 0,92	21.72a ± 0,11	11.43a ± 0,07
	"Marcela" 0.5%	47.95a ± 0,13	19.04a ± 1,42	11.06a ± 0,33
	"Marcela" 1%	49.71a ± 0,22	18.81a ± 0,12	11.34a ± 0,12
14	Control	44.26a ± 0,69	24.01a ± 0,41	11.22a ± 0,04
	"Marcela" 0.5%	43.28a ± 0,88	21.26a ± 0,28	10.87a ± 0,25
	"Marcela" 1%	42.11a ± 0,33	21.58a ± 0,91	11.60a ± 0,21
19	Control	37.78a ± 0,81	24.33a ± 0,82	10.59a ± 0,04
	"Marcela" 0.5%	42.55a ± 0,62	22.28a ± 0,45	10.82a ± 0,05
	"Marcela" 1%	38.02a ± 1,77	21.63a ± 0,82	11.03a ± 0,11

Means followed by the same letter, at the same column, at the same day, did not show significant difference ($p \leq 0.05$) in the Tukey test. * Control: without addition of "Marcela" extract; "Marcela" 0.5% : addition of 0.5% of hydroalcoholic extract of "Marcela"; "Marcela" 1%: addition of 1% of hydroalcoholic extract of "Marcela".

Table 2. Mean values of TBARS numbers (± standard deviation) during the storage of salami formulated with different levels of hydroalcoholic extract of "Marcela".

Days	Control	"Marcela" 0.5%	"Marcela" 1%
0	$0,546^{a} \pm 0,001$	$0,405^{b} \pm 0,002$	$0,305^{c} \pm 0,001$
15	$0,604^{a} \pm 0,003$	$0,429^{b} \pm 0,004$	$0,312^{c} \pm 0,006$
30	$0,702^{a} \pm 0,001$	$0,507^{b} \pm 0,004$	$0,429^{c} \pm 0,004$
45	$0,819^{a} \pm 0,004$	$0,682^{b} \pm 0,007$	$0,351^{c} \pm 0,004$
60	$0,526^{a} \pm 0,003$	$0,448^{ab} \pm 0,007$	$0,331^{b} \pm 0,007$
75	$0,546^{a} \pm 0,006$	$0,351^{b} \pm 0,004$	$0,390^{b} \pm 0,006$
90	$0,507^{a} \pm 0,004$	$0,390^{b} \pm 0,009$	$0,370^{b} \pm 0,009$

Means followed by the same letter, at the same column, at the same day, did not show significant difference ($p \leq 0.05$) in the Tukey test.* Control: without addition of "Marcela" extract; "Marcela" 0.5% : addition of 0.5% of hydroalcoholic extract of "Marcela"; "Marcela" 1%: addition of 1% of hydroalcoholic extract of "Marcela".

were followed during the 90 days of storage (Table 2). The lipidic oxidation was significantly affected ($p < 0.05$) by the addition of "Marcela" extract. At the beginning of the storage (0 day), the TBARS values of the treatments containing 0.5 and 1% of "Marcela" extract presented a decrease of 26% and 44%, respectively, in relation to the control, which presented significantly lower values than those of the control during the whole period of storage. After 45 days of storage, the TBARS number in the treatment containing 1% of "Marcela" extract was half the value found in the control. Those results suggest that the "Marcela" extract retarded the lipidic oxidation during the storage period of the salami. It was observed in this experiment, for all treatments, an increase in the TBARS values up to the 45th day of storage followed by a decrease. This decrease may be attributed to the reactions of malonaldeyde with proteins during the storage period (SAMMET et al., 2006). Nassu et al. (2003) in a study evaluating the effect of different levels of rosemary

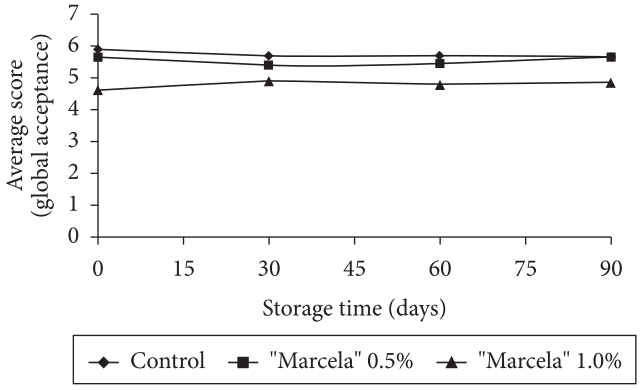

Figure 3. Average scores of global acceptance during the storage of salami formulated with different levels of hydro-alcoholic extract of "Marcela". Control: without addition of "Marcela" extract; "Marcela" 0.5%: addition of 0.5% of hydro-alcoholic extract of "Marcela"; "Marcela" 1%: addition of 1% of hydro-alcoholic extract of "Marcela".

(*Rosmarinus officinalis*) on the oxidative stability of fermented sausage of caprine, reported TBARS values similar to those found in this study during a storage period of 90 days. Similar results have also been reported by Bozkurt (2006) in a study on the effect of different antioxidants on Turkish salami ("sucuk") during its production.

3.2 Sensorial analysis

The average scores of global acceptance as a function of storage time are presented in Figure 3. According to Labuza and Schmidl (1988), the end of the life cycle of the product occurs when there is a decrease of 1.5 points at the hedonic scale. In this experiment, this did not occur for any sample indicating that all treatments may be considered acceptable up to 90 days of storage at room temperature. No significant differences were found between the treatment containing 0.5% of "Marcela" extract and the control. However, the addition of 1% of hydroalcoholic extract of "Marcela" decreased significantly the global acceptance depreciating the sensorial quality of the product.

4 Conclusion

The hydroalcoholic extract of "Marcela" was effective in decreasing the lipidic oxidation of salami during storage. The addition of 0.5% of hydroalcoholic extract of "Marcela" did not interfere in the global acceptance of the product. Therefore, this concentration may be used in salami elaboration providing safer products for the consumers.

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