Original

Rosemary extract and celery-based products used as natural quality enhancers for colonial type salami with different ripening times

Extrato de alecrim e produtos derivados do aipo como agentes naturais potencializadores da qualidade de salames coloniais com diferentes tempos de maturação

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Abstract

This study aimed to evaluate the use of rosemary (*Rosmarinus officinalis*) extract (RE), celery (*Apium graveolis*), and low levels of NO_3 and NO_2 as natural agents to enhance the quality of colonial salami. Salami was produced according to three treatments: (A) Control: 0.1% curing salt; (B) Rosemary: 0.05% curing salt + 0.5% RE (rosemary extract); and (C) Rosemary+celery: 0.14% Veg 503 + 0.27% Veg 504 (sea salt plus celery) + 0.5% of RE (rosemary extract). There was no effect ($P > 0.05$) of the treatments on water activity, Na content, and residual NO_3 and NO_2. Fatty acids C18:2 and C20:4 were reduced ($P < 0.05$) during the ripening period in the Control treatment indicating possible oxidation. The use of celery resulted in lower pH values ($P < 0.05$) in the salami. Reduced addition of NO_3 and NO_2 resulted in salami lighter in color (higher L* values, $P < 0.05$) at the 12th day of ripening. In conclusion, celery-based products proved to be an effective source of NO_2 and NO_3 for color development, but the low pH of the product indicates the need for better evaluation of its use in fermented salami. The RE (rosemary extract) reduced fat oxidation in salami, but this needs further evaluation.

Keywords: *natural preservatives; colonial type meat products; vegetable extracts.*

Resumo

Objetivou-se avaliar o efeito do extrato de alecrim (EA; *Rosmarinus officinalis*) e do aipo (*Apium graveolis*) e de baixos níveis de adição de NO_3 e NO_2, como agentes naturais potencializadores da qualidade dos salames coloniais. Foram produzidos salames de acordo com três tratamentos: (A) Controle: 0,1% de sal de cura; (B) Alecrim: 0,05% de sal de cura + 0,5% de EA; (C) Alecrim+aipo: 0,14% de Veg 503 + 0,27% de Veg 504 (sal marinho e aipo) + 0,5% de EA. Não houve efeito ($p > 0,05$) dos tratamentos sobre o conteúdo de Na, atividade de água e NO_3 e NO_2 residuais. Houve redução ($p < 0,05$) dos ácidos graxos C18:2 e C20:4 durante o período de maturação no tratamento Controle, indicando sua possível oxidação. O uso do aipo resultou em baixo ($p < 0,05$) pH no salame. A redução da adição de NO_3 e NO_2 resultou em salames com coloração mais clara (valores de L* mais elevados, $p < 0,05$) aos 12 dias de maturação. Conclui-se que o aipo foi efetivo como fonte de NO_3 e NO_2 para desenvolvimento da cor, mas o baixo pH do produto indica a necessidade de melhor avaliar sua utilização em salames fermentados. Os salames produzidos com EA poderão apresentar diferencial de qualidade pela menor oxidação das gorduras, mas isto necessita ser confirmado em estudo futuros.

Palavras-chave: *conservantes naturais; embutidos coloniais; extratos vegetais.*

1 Introduction

Homemade products derived from pork, such as salami, are traditionally consumed by members of family farming systems in Southern Brazil and are popularly known as homemade rural products. In addition to the homemade system, the colonial type salami is also produced by small agribusiness. In the context of agro-industrialization, for both, family farming systems and small or large agribusiness, the tendency is to continually add value to these products considering that pig farming and pork processing are a source of income for social and economic development in Southern Brazil (ESPÍNDOLA, 2002).

An alternative to add value to the colonial type salami is to reduce the use of chemical additives while maintaining safety and the quality of the product with regard to the microbiological, sensory, and physicochemical aspects. The replacement of chemical additives such as antioxidants (2-t-butyl-4-methoxyphenol, BHA, and 2,6-di-t-butyl-4-methylphenol, BHT, etc.), color enhancers, and control agents of undesirable microorganism/pathogen (containing nitrite and/or nitrate curing salt) with natural products is a promising alternative.

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A total of 30% fat is added to the original composition of salami, which is susceptible to rancidity and oxidation.

The compounds resulting from fat deterioration can compromise product quality resulting in unpleasant odor, discoloration and dehydration, and potential risk to human health due to the production of toxic or toxigenic substances (MORRISSEY et al., 1998). Therefore, antioxidants are used in order to promote product quality to control free radicals and oxidation of fats and other compounds of meat mixture. Regarding the control of pathogenic microorganism, the Brazilian legislation (BRASIL, 2000) requires the addition of curing salt (sodium or potassium nitrate and nitrite) in the formulation of raw meat products. Another important function of curing salts is the development of red color, characteristic of fermented raw meat products.

Some plant extracts have been evaluated for their properties as natural antioxidants (YEN; CHEN, 1995; LOPEZ-BOTE et al., 1998; LEE; SHIBAMOTO, 2002; BASMACIOGLU; TOKUSOGLU; ERGUL, 2004; RABABAH et al., 2004), which is mainly attributed to the flavonoids present in the plants. Some extracts were classified by Lee and Shibamoto (2002) according to their antioxidant power in the following order: thyme (*Thymus vulgaris*) > basil (*Ocimum basilicum L.*) > rosemary (*Rosmarinus officinalis*) > chamomile (*Matricaria recutita*) > lavender (*Lavandula* spp.) > cinnamon (*Cinnamomum zeylanicum*).

The antibacterial and antioxidant effect of rosemary, evaluated *in vitro*, was investigated by Asolini, Tedesco and Carpes (2006) and Klancnik, Guzej, and Kolar (2009), who observed that the result was dependent on the concentration of solute and solvent used. Its antioxidant effect on meat products was investigated by Basmacioglu, Tokusoglu and Ergul (2004) and Riznar et al. (2006). Although the antioxidant properties of various plants and herbs have been studied *in vitro* and in foodstuffs, there are no reports of studies on salami.

Celery (*Apium graveola*) is a leafy plant with significant levels of nitrites and nitrates (SANTAMARIA, 2006); it is a possible natural source of these compounds for use in cured/fermented products. Its effects in cooked and cured products were studied by Sindelar et al. (2007a, b), who demonstrated the potential for such use although additional studies are needed.

Currently, rosemary oil extract and celery-based products are commercially available for use as food additives, thus allowing their regular use in the manufacture of processed products. Considering that these additives are natural extracts, this study evaluated the effect of rosemary oil extract, celery-based products, and low levels of added sodium nitrate and nitrite on the microbiological and physicochemical characteristics and on the oxidation of salami, characterized as colonial type salami.

2 Material and methods

2.1 Salami preparation

The pork meat cuts for salami production were obtained from animals from the Embrapa Swine and Poultry (Concórdia, Santa Catarina State, Brazil), slaughtered at an abattoir with municipal sanitary inspection. The pork used was composed of 60% ham, 30% shoulder, and 10% back-fat, all ground uniform in size. Deboning of carcasses was performed 24 hours after slaughter, and the meat was frozen for 48 hours. After this period, meat and fat were ground, and the ingredients were added according to three treatments: (A) Control: Addition of 0.1% curing salt; (B) Rosemary: Addition of 0.05% curing salt + 0.5% deodorized rosemary oil extract; and (C) Rosemary + celery: Addition of 0.14% Veg 503 + 0.27% Veg 504 from the compound celery powder and sea salt + 0.5% deodorized rosemary oil extract. The formulation used in each treatment is shown in Table 1. The mixture remained at rest under refrigeration (0-4 °C) for 2 hours. The sausages were manufactured at the Federal Institute of Education, Science and Technology of Santa Catarina - Campus Concórdia, Concórdia, Santa Catarina State. Four replicates (batches) were performed for each treatment.

A portion of approximately 350 g of the mixture was stuffed into synthetic cellulose casings (55 mm diameter), smoked at 25 °C for 8 hours, and ripened under controlled humidity for 12 days. Afterwards, the salami was stored under room temperature and humidity until the end of the sampling period (30 days).

At 0, 6, 12, 18, 24, and 30 days of ripening, the salami samples were randomly gathered for microbiological, physicochemical and fatty acids analyses. All tests were performed in replicate.

The extract of rosemary and Veg stable compounds (503 and 504), both formulated with dehydrated celery and sea salt, were purchased from commercial companies.

2.2 Microbiological analyses

Microbiological analyses of salami were performed on samples collected at 0, 6, 12, and 30 days of ripening, according

Table 1. Formulation used in the preparation of salami in each treatment.

Raw material %	Treatment		
	(A) Control	(B) Rosemary	(C) Rosemary + Celery
Pork[a]	95.77	95.27	94.91
Salt (NaCl)	2.91	2.96	2.96
Curing salt[b]	0.1	0.05	-
Celery powder Veg 503[c]	-	-	0.14
Celery powder Veg 504[d]	-	-	0.27
Nutmeg	0.03	0.03	0.03
Black pepper powder	0.2	0.2	0.2
Wine	0.26	0.26	0.26
Garlic	0.2	0.2	0.2
Rosemary extract[e]	-	0.5	0.5
Sugar	0.5	0.5	0.5
Starter culture[f]	0.025	0.025	0.025

[a]Containing 60% ham, 30% shoulder and 10% back-fat; [b]Containing 85% NaCl, 9% NO_2 and 6% NO_3 from sodium nitrite and nitrate, respectively; [c]Containing 3.0 to 3.3% NO_3 – Florida Food Products, INC.; [d]Containing 1.0 to 1.2% NO_2 - Florida Food Products, INC.; [e]Rosemary oil extract - Fuchs Gewürze do Brasil Ltda; [f]Provided 5 × 10^6 CFU *Staphylococcus xylosus* and 2.5 × 10^6 CFU *Lactobacillus plantarum*/g of mixture (Lyocarni SHA-24, Sacco Brasil).

to APHA (AMERICAN..., 1992). The analyses consisted of the estimated count of the population of lactic acid bacteria, Gram-positive cocci, and catalase-positive, as well as detection of spoilage and pathogenic microorganisms.

Twenty-five grams of each sample were added to 225 mL of sterile 0.1% peptone water (w/v) (Oxoid) and homogenized in a stomacher (ITR, model MK 1204) for 2 minutes. Subsequent decimal dilutions were prepared using the same diluent, and the sample with appropriate dilution was inoculated into selective medium and incubated under specific conditions: De Man, Rogosa and Sharp Agar - MRS (Oxoid) for lactic acid bacteria (30 °C/72 hours), the plates were placed in an anaerobic jar (Anaerojar Assembly - CODE AG25 - Oxoid) with CO_2 enriched air (AN Anaerogen 2.5 L - Oxoid); Brain Heart Infusion Agar - BHI (Oxoid) for *Staphylococcaceae* (35 °C/48 hours); Plate Count Agar - PCA (Oxoid) for total aerobic mesophilic bacteria (30 °C/72 hours); Brilliant Green Bile (2%) broth - BGBB (Oxoid) for total coliforms (method NMP at 37 °C/48 hours); EC broth (Oxoid) for thermotolerant coliforms (MPN method at 42 °C/24 hours); Baird-Parker Agar (Oxoid), plus egg and tellurite emulsion (Oxoid) for *Staphylococci* coagulase - positive (at 37 °C/48 hours); and Perfringens Agar Base - TSC (Oxoid).

For the detection of *Salmonella* ssp, pre-enrichment was performed by suspending 25 g in 225 mL of BPW (1%) (w/v) (Merck) and incubating at 37 °C/16 hours Selective enrichment was performed by transferring 0.1 mL pre-enriched medium to 10 mL of Rappaport-Vassiliadis (Merck) and to 10 mL of Selenite Cystine broth (Oxoid), respectively. Next, all tubes were incubated at 42 °C/24 hours. After incubation, each selective broth was streaked onto enriched modified BPLS agar (Merck) and XLD agar (Merck), and all plates were incubated at 37 °C for 18-24 hours.

For detection of *Listeria* spp, selective enrichment was performed by adding 25 g of sample to 225 mL of Listeria selective enrichment broth (Merck) and incubating at 30 °C/24 hours. For a secondary enrichment, 0.1 mL pre-enrichment broth was transferred to Fraser broth (Merck) and incubated at 35 °C for 48 hours. Then, a loop full of enriched culture was streaked onto PALCAM agar (Merck) and incubated at 30 °C/48 hours and on Oxford agar (OA) and incubated at 360 °C/24 hours.

The results were expressed as colony forming units per gram of sample (CFU. g^{-1}) or most probable number per gram of sample (MPN.g^{-1}).

2.3 Physicochemical and color analyses

Water activity, NO_2, and NO_3 were analyzed at 0, 6, 24, and 30 days of ripening. Na content was analyzed on the 12th and 30th days of ripening, while the pH was measured at 0, 6, 12, 18, 24, and 30 days. Color measurements (L*, a*, and b*) were carried out at 12, 18, 24, and 30 days of ripening.

Sodium was analyzed in the dry sample according to the methodology of AOAC (ASSOCIATION..., 1995). The sample was digested with a mix of nitric and perchloric concentrated acids, at high temperature (210 °C). Sodium reading was taken on a flame photometer.

The pH was measured according to AOAC (ASSOCIATION..., 1995). Water activity (Aw) was determined using a digital Testo 400 CE device (Testo GmbH & Co., Brazil).

The CIE Lab (L*, a*, b*) system was used for color measurements that were performed using a Minolta colorimeter (Minolta Camera Ltd., Japan) in the range of 400 to 700 nm. The L* value measures light reflectance, and in the color scale [in a scale from 0 (black) to 100 (white)], lower L* values indicate darker color and higher values indicate lighter color of the product. Positive a* is red and negative a* is green, while positive b* is yellow and negative b* is blue. The sample was cross-sectioned at both extremities, and color measurements were done after exposing the surfaces to air for 20 minutes. The mean values of both measurements were submitted to statistical analysis. Saturation index, which indicates the color intensity, was calculated using the Equation 1 (LITTLE, 1975):

$$Saturation\ index = \sqrt{\left(a^{*2} + b^{*2}\right)} \quad (1)$$

Sample preparation for the assessment of nitrite and nitrate was done according to AOAC (ASSOCIATION..., 1995) and determined using flow injection spectrophotometric analysis (FiaLab2500)

2.4 Fatty acids

Fatty acids were analyzed at 0, 6, 12, 18, 24, and 30 days of ripening. The samples were prepared by the saponification and esterification method of Hartman and Lago (1973) after extraction of total lipids by Folch, Less, and Stanley (1957). Fatty acid methyl esters (FAME's) were analyzed by injecting (1 µL) of sample solution into a Varian CP-3800 gas chromatography system (Walnut Creek, CA, USA Palo Alto, USA), equipped with a split/splitless injector (1:100), a CP Sil 88 capillary column (50 m × 0.25 mm i.d. × 0.2 µm film thickness), a flame ionization detector (FID), and a Varian CP 8410 auto sampler. Oven temperature was set to rise from 80 °C to 150 °C at 5 °C/min, then from 150 °C to 220 °C at 2 °C/min, and held at 220 °C for 6 minutes. The injector and detector temperatures were fixed at 240 °C and 280 °C, respectively. Nitrogen was used as carrier gas at 1 mL/min. FAME peaks were identified by comparison of samples' retention times with authentic standards (Sigma Chemical Co., St. Louis, MO, USA). Peak area normalization method was used for quantification.

2.5 Statistical analysis

The data were analyzed using the MIXED procedure of SAS for repeated measures (STATISTICAL..., 2001) considering the effect of treatment and block (batch) as the main plot, ripening time as the secondary plot, and the interaction between treatment and ripening time as sources of variation. Sixteen matrices of variance and covariance were evaluated, and the matrix that best fit the data was identified considering the Akaike's Information Criterion (AIC), according to XAVIER (2000). The restricted maximum- likelihood estimation method

was used. Treatment mean values were compared by Tukey's test ($P < 0.05$).

3 Results and discussion

3.1 Microbiological analyses

All analyzed samples of salami met the microbiological standards set by the Brazilian health legislation for ripened meat products (Table 2; BRASIL, 2001). No potential risk was verified regarding the presence of spoilage, pathogenic, and/or toxigenic microorganisms. These results indicate a reduced contamination by undesirable microorganisms in meat products with good conditions of hygiene and good manufacturing practices.

The evolution of homofermentative lactic acid bacterial population found in the samples of salami during fermentation and ripening demonstrates that the population of these organisms evolved on average two log cycles during the first twelve days of ripening in all treatments. Regarding the population of Gram-positive and catalase-positive, it was observed that the microbial population remained constant up to the twelfth day in all treatments. These results showed that the starter culture added to the product was not inhibited by intrinsic factors of the respective treatments. This result is

Table 2. Estimation of microbial population in salami according to treatment and ripening time.

Microorganism	Ripening time (days)	Treatment			Pr > F
		Control	Rosemary	Rosemary + celery	Treatment
Lactic acid bacteria (Log CFU.g^{-1})	0	5.98	5.93	6.23	0.63
	6	7.78	7.93	8.19	0.45
	12	7.47	7.95	8.19	0.10
	30	6.72	7.08	7.07	0.46
Gram-positive, catalase-positive cocci (Log CFU.g^{-1})	0	5.74	5.52	5.60	0.32
	6	5.24	5.38	5.55	0.52
	12	5.12	5.33	4.87	0.24
	30	5.06[a]	4.89[a]	4.52[b]	0.01
Total aerobic mesophilic (Log CFU.g^{-1})	0	5.45	5.61	5.68	0.46
	6	5.61	5.46	5.28	0.90
	12	5.17	5.04	5.07	0.97
	30	5.37	5.09	5.25	0.89
Total coliform (MPN.g^{-1})	0	<1	<1	<1	-
	6	<1	<1	<1	-
	12	<1	<1	<1	-
	30	<1	<1	<1	-
Thermotolerant coliforms (MPN.g^{-1})	0	<1	<1	<1	-
	6	<1	<1	<1	-
	12	<1	<1	<1	-
	30	<1	<1	<1	-
Staphylococci coagulase - positive (Log CFU.g^{-1})	0	<1	<1	<1	-
	6	<1	<1	<1	-
	12	<1	<1	<1	-
	30	<1	<1	<1	-
Salmonella spp.	0	Absent	Absent	Absent	-
	6	Absent	Absent	Absent	-
	12	Absent	Absent	Absent	-
	30	Absent	Absent	Absent	-
Listeria spp.	0	Absent	Absent	Absent	-
	6	Absent	Absent	Absent	-
	12	Absent	Absent	Absent	-
	30	Absent	Absent	Absent	-

[a,b]Means followed by different letters on the row are significantly different ($p < 0.05$) by Tukey's test.

important because starter cultures can provide microbiological, physicochemical, and sensory characteristics desired in meat products (RANTSIOU et al. 2005; LEROY; VERLUYTEN; VUYST, 2006; FIORENTINI, 2008; SAWITZKI et al., 2008).

3.2 Physicochemical and color analyses

Water activity and Na concentration were not affected by the treatments ($p > 0.05$), but water activity decreased ($p < 0.01$) and Na concentration increased ($p < 0.01$) over ripening time (Table 3). Water activity values remained below 0.90 starting at day zero and dropped sharply within the first 12 days of ripening, which means they fell below the standards set by legislation for Milano or Italian type salami (BRASIL, 2000). The water activity of salami in the present study was within safe limits to prevent the development of pathogenic microorganisms. Values of water activity in the present study are lower than those reported by Cichoski, Zis and Franceschetto (2009) in Italian type salami produced with similar content of sodium chloride.

It was observed a consistent effect of treatments on the pH over ripening time. In the Control and Rosemary treatments, the pH decreased ($p < 0.01$) up to day 12, and after that it remained almost constant. In the Rosemary + celery treatment, the pH remarkably decreased ($p < 0.01$) by day six, remaining stable after that. Consequently, from day six of ripening, salami containing rosemary + celery showed lower pH ($p < 0.05$) than that of the other treatments.

For all treatments, there was a substantial drop in pH on the first six days of ripening compared to the pH values in the rest of ripening period. The initial rapid reduction in pH can be assigned to the production of lactic acid by *Lactobacillus plantarum* (FIORENTINI et al., 2001) present in the starter culture added in all treatments. Rosemary extract did not cause pH changes during the ripening period, but celery-based products accentuated the initial pH decrease maintaining the values lower than those of the other two treatments up to the end of ripening. Although the antimicrobial effect of rosemary has been proven *in vitro* and when applied to products (RIZNAR et al. 2006; KLANCNIK; GUZEJ; KOLAR, 2009; SHAN et al., 2009), this effect seems not to have occurred on the bacteria from the starter culture in the present study. The lower pH values observed in the salami containing rosemary + celery can be the result of various factors such as the presence of evaporated sugar cane juice in Veg 503, which supplied an additional source of sucrose to *L. plantarum* for lactic acid production, and/or a possible stimulating effect of celery on the activity of these microorganisms. The Control and Rosemary treatments had final pH close to that expected for Italian type salami produced in Brazil, from 5.2 to 5.4, while in the Rosemary + Celery treatment, the pH was lower than expected (TERRA, 2006).

Table 3. Mean values and standard errors obtained for physicochemical characteristics of salami according to treatment and ripening time.

Dependent variables[a]	Ripening time (days)	Treatment			Pr > F
		Control	Rosemary	Rosemary + Celery	Treatment
Water activity	0	0.833 ± 0.010^{A}	0.840 ± 0.014^{A}	0.837 ± 0.021^{A}	0.95
	6	0.772 ± 0.008^{B}	0.748 ± 0.010^{B}	0.784 ± 0.004^{B}	0.21
	12	0.715 ± 0.005^{C}	0.723 ± 0.011^{B}	0.728 ± 0.014^{C}	0.55
	30	0.672 ± 0.013^{C}	0.588 ± 0.029^{C}	0.621 ± 0.032^{D}	0.12
Pr > F Ripening time		0.001	0.001	0.001	
Na, $mg.kg^{-1}$	12	14006 ± 450^{B}	12535 ± 1154^{B}	13540 ± 570^{B}	0.12
	30	17948 ± 451^{A}	17166 ± 889^{A}	16702 ± 636^{A}	0.20
Pr > F Ripening time		0.001	0.001	0.001	
pH	0	5.84 ± 0.05^{A}	5.83 ± 0.06^{A}	5.82 ± 0.08^{A}	0.92
	6	5.23 ± 0.09^{Ba}	5.20 ± 0.12^{Ba}	4.95 ± 0.12^{Bb}	0.001
	12	5.09 ± 0.17^{Ca}	5.02 ± 0.161^{Ca}	4.89 ± 0.14^{Bb}	0.01
	18	5.21 ± 0.08^{BCa}	5.21 ± 0.12^{BCa}	4.98 ± 0.09^{Bb}	0.02
	24	5.18 ± 0.08^{BCa}	5.14 ± 0.10^{BCa}	4.87 ± 0.08^{Bb}	0.001
	30	5.18 ± 0.08^{BCa}	5.15 ± 0.12^{BCa}	4.92 ± 0.08^{Bb}	0.01
Pr > F Ripening time		0.001	0.001	0.001	
NO_2, $mg.kg^{-1}$	0	14.80 ± 3.59^{A}	9.56 ± 1.16	6.97 ± 1.02	0.10
	6	2.20 ± 0.52^{B}	3.98 ± 1.10	3.51 ± 1.19	0.67
	12	1.86 ± 0.13^{B}	3.65 ± 0.92	3.47 ± 0.87	0.12
	30	2.94 ± 0.83^{bB}	3.79 ± 1.06^{a}	3.64 ± 0.95^{a}	0.01
Pr > F Ripening time		0.001	0.10	0.46	
NO_3, $mg.kg^{-1}$	0	117.58 ± 35.5	82.64 ± 32.80	100.73 ± 31.00	0.09
	6	101.11 ± 10.20	81.10 ± 9.57	86.80 ± 15.90	0.93
	12	108.45 ± 14.00	72.34 ± 11.30	86.47 ± 12.90	0.72
	30	117.98 ± 17.10	92.94 ± 19.10	106.38 ± 16.20	0.87
Pr > F Ripening time		0.71	0.11	0.16	

[a,b,c,d]Means followed by different lower case on the row, and different capital letters in columns are significantly different ($p < 0.05$) by Tukey's test.

The NO_2 content decreased with ripening time, but only in the Control treatment the differences were statistically significant ($p < 0.001$) (Table 3). The Control treatment had lower ($p < 0.01$) NO_2 than the other treatments at day 30; however, this difference is of minor importance given the low magnitude of the values. Residual NO_3 remained constant during ripening and was not affected by the treatments ($p > 0.05$). The products derived from celery used in this study as sources of NO_3 and NO_2 proved to be effective, considering that residual values of NO_3 and NO_2 in salami were similar to the values observed in the salami added with curing salt.

Very low values of NO_2 were obtained starting at day zero in all treatments and are considerably lower than the residual levels found in commercial products (SOUZA; FALEIROS; SOUZA, 1990; MELO FILHO; BISCONTINI; ANDRADE, 2004). The storage of samples and smoking process may have sped up the conversion of NO_2 to NO and to nitrosyl myoglobin, a possible explanation for the low values found.

The results are consistent with reports of Sindelar et al. (2007b), who observed constant values of NO_3 during the ripening period of cooked sausages to which only NO_2 was added. Sindelar et al. (2007a) observed reduced levels of NO_3 in cooked hams that were not subjected to incubation, but not in those that were incubated.

The reduction of NO_3 to NO_2 is accelerated by the presence of nitrate-reducing bacteria such as *S. xylosus* (PINTO; PONSANO; HEINEMANN, 2001) present in the starter culture used in the present study. However, this process did not take place satisfactorily. High levels of NO_3 and low levels of NO_2 also indicate a re-conversion of NO_2 to NO_3, which may be related to the preservation of samples by chilling until lyophilization and analysis. Moreover, the reduction of NO_2 to NO is sped up by NaCl (FOX JUNIOR, 1987) and acidifying agents such as lactic acid bacteria (KALLE, 1955 apud FOX JUNIOR, 1987), also present in the starter culture. This is another possible explanation for the low levels of NO_2 observed in the present study. Other authors have found low levels of residual nitrite in salami prepared with the addition of *L. plantarum* (SAWITZKI et al., 2007) or *S. xylosus* (FIORENTINI, 2008) as starter cultures.

When the values of $NO_2 + NO_3$ observed at the thirtieth day of ripening are converted to the same dry matter basis found at day zero (33% dry matter at day zero and 66% at day 30), it was observed a reduction of approximately 60% of the initial concentration of $NO_3 + NO_2$ in the Control treatment, 35% in the Rosemary treatment, and 27% in the Rosemary + celery treatment. This indicates that the extracts may have somehow interfered with the transformation of NO_2 and NO_3 to other chemical forms.

The levels of NO_2 and NO_3 added to salami in the present study were relatively low considering the levels allowed by Brazilian legislation, which sets no minimum or maximum threshold of NO_2 and NO_3 in food products, but it does the maximum residual NO_2 and NO_3 (BRASIL, 1998), which are quite high compared with other countries' legislation.

Regarding the color, at the 12th day, Control treatment salami had lower ($p < 0.001$) L* values indicating darker color (Table 4). At later ripening times, there was no difference between the treatments. L* values decreased from day 12 to day 18 in the Rosemary and Rosemary + celery treatments

Table 4. Mean values and standard errors obtained for color evaluation in salami according to treatment and ripening time.

Dependent variables	Ripening time (days)	Treatment			Pr > F treatment
		Control	Rosemary	Rosemary + Celery	
L*	12	46.70 ± 2.04^{b}	51.31 ± 2.04^{Aa}	53.50 ± 1.67^{Aa}	0.001
	18	46.24 ± 2.36	49.05 ± 2.72^{AB}	48.80 ± 1.72^{B}	0.06
	24	46.91 ± 2.73	48.99 ± 2.70^{AB}	48.56 ± 2.10^{B}	0.18
	30	47.61 ± 1.29	47.84 ± 2.00^{B}	49.18 ± 2.20^{AB}	0.35
Pr > F Ripening time		0.46	0.05	0.001	
a*	12	13.35 ± 0.59^{B}	13.50 ± 0.97	13.30 ± 0.64	0.94
	18	14.62 ± 0.12^{A}	14.60 ± 0.49	14.40 ± 0.23	0.92
	24	15.47 ± 0.42^{AB}	16.08 ± 0.86	14.78 ± 0.80	0.12
	30	16.33 ± 0.62^{A}	15.13 ± 0.63	14.97 ± 0.42	0.07
Pr > F Ripening time		0.05	0.08	0.34	
b*	12	3.09 ± 0.62^{Bb}	5.65 ± 0.85^{a}	6.51 ± 0.69^{Aa}	0.001
	18	2.73 ± 0.47^{Bb}	5.46 ± 0.66^{a}	5.20 ± 0.63^{BCa}	0.001
	24	3.97 ± 0.83^{A}	5.42 ± 1.08	4.93 ± 1.21^{C}	0.78
	30	4.43 ± 1.47^{Ab}	4.11 ± 1.63^{b}	5.57 ± 1.78^{ABCa}	0.001
Pr > F Ripening time		0.001	0.62	0.001	
Color saturation	12	13.73 ± 0.59^{C}	14.68 ± 1.14	14.84 ± 0.79	0.16
	18	14.89 ± 0.17^{BC}	15.64 ± 0.37	15.34 ± 0.31	0.58
	24	16.04 ± 0.24^{ABb}	17.08 ± 0.53^{a}	15.74 ± 0.50^{b}	0.04
	30	17.08 ± 0.67^{A}	15.86 ± 0.48	16.18 ± 0.31	0.15
Pr > F Ripening time		0.01	0.06	0.73	

[a,b,c] Means followed by different lower case on the row, and different capital letters in columns are significantly different ($p < 0.05$) by Tukey's test.

Table 5. Mean values and standard errors obtained for quantification of fatty acids in salami according to treatment and ripening time.

Fatty acid, % of fat	Ripening time (days)	Treatment			Pr > F treatment
		Control	Rosemary	Rosemary + Celery	
Total saturated	0	37.62 ± 0.58^{a}	36.48 ± 0.38^{b}	36.30 ± 0.62^{b}	0.01
	6	37.04 ± 0.77	37.06 ± 0.68	37.39 ± 0.36	0.92
	12	38.41 ± 0.72	36.44 ± 1.25	37.82 ± 1.69	0.49
	18	36.94 ± 0.77	36.25 ± 0.33	36.01 ± 0.82	0.65
	24	35.88 ± 0.69	34.10 ± 1.06	36.30 ± 0.62	0.42
	30	37.70 ± 0.39^{a}	36.45 ± 0.63^{b}	36.58 ± 0.55^{b}	0.001
Pr>F Ripening time		0.66	0.71	0.48	
C16:1	0	2.052 ± 0.062^{A}	2.005 ± 0.078	2.041 ± 0.071^{A}	0.48
	6	2.013 ± 0.073^{ABa}	1.934 ± 0.069^{b}	1.910 ± 0.082^{Bb}	0.04
	12	1.970 ± 0.042^{A}	1.893 ± 0.037	1.878 ± 0.119^{AB}	0.12
	18	1.933 ± 0.096^{B}	1.937 ± 0.079	1.933 ± 0.104^{B}	0.99
	24	1.927 ± 0.090^{AB}	1.861 ± 0.104	1.920 ± 0.042^{AB}	0.45
	30	1.993 ± 0.075^{AB}	1.962 ± 0.092	1.942 ± 0.082^{B}	0.45
Pr>F Ripening time		0.02	0.28	0.01	
C18:1	0	40.75 ± 1.34	40.62 ± 1.17	41.15 ± 0.81	0.39
	6	40.30 ± 1.02	40.20 ± 1.25	40.15 ± 1.14	0.96
	12	39.23 ± 1.32	39.88 ± 0.59	39.32 ± 1.37	0.79
	18	40.09 ± 1.82	40.22 ± 1.31	40.26 ± 1.74	0.99
	24	38.77 ± 2.66	38.46 ± 3.03	40.14 ± 1.18	0.54
	30	41.49 ± 0.93^{a}	40.45 ± 1.01^{b}	40.57 ± 1.08^{b}	0.01
Pr>F Ripening time		0.07	0.92	0.30	
C18:2	0	13.78 ± 1.26^{A}	14.42 ± 1.00^{B}	14.59 ± 1.05	0.11
	6	13.99 ± 0.85^{Ab}	14.35 ± 0.96^{Ba}	14.32 ± 0.97^{a}	0.05
	12	13.68 ± 1.06^{Bb}	15.05 ± 0.61^{ABa}	14.86 ± 0.48^{a}	0.001
	18	11.84 ± 0.48^{BC}	13.45 ± 0.99^{AB}	14.14 ± 0.87	0.17
	24	12.51 ± 0.04^{Cc}	15.16 ± 0.13^{Ab}	15.97 ± 0.45^{a}	0.001
	30	12.19 ± 0.67^{Bb}	14.17 ± 0.89^{ABa}	14.28 ± 0.89^{a}	0.001
Pr>F Ripening time		0.001	0.05	0.71	
C18:3	0	1.532 ± 0.203^{A}	1.518 ± 0.147^{A}	1.483 ± 0.156^{A}	0.87
	6	1.448 ± 0.197^{AC}	1.472 ± 0.206^{AC}	1.449 ± 0.227^{AC}	0.97
	12	1.495 ± 0.230^{ABC}	1.529 ± 0.174^{AC}	1.549 ± 0.180^{AC}	0.39
	18	1.079 ± 0.064^{D}	1.209 ± 0.102^{CD}	1.170 ± 0.178^{CD}	0.75
	24	1.444 ± 0.006^{BCD}	1.506 ± 0.149^{BCD}	1.332 ± 0.173^{BCD}	0.54
	30	1.250 ± 0.109^{CD}	1.041 ± 0.033^{D}	1.045 ± 0.044^{D}	0.33
Pr>F Ripening time		0.001	0.001	0.001	
C20:2	0	0.640 ± 0.030^{CD}	0.602 ± 0.038	0.616 ± 0.054	0.31
	6	0.631 ± 0.051^{CDa}	0.602 ± 0.041^{b}	0.607 ± 0.044^{b}	0.01
	12	0.721 ± 0.012^{BC}	0.641 ± 0.031	0.667 ± 0.054	0.12
	18	0.626 ± 0.046^{D}	0.644 ± 0.067	0.603 ± 0.035	0.60
	24	0.899 ± 0.120^{Aa}	0.654 ± 0.012^{b}	0.681 ± 0.010^{b}	0.001
	30	0.819 ± 0.139^{AB}	0.650 ± 0.092	0.639 ± 0.058	0.17
Pr>F Ripening time		0.001	0.50	0.92	
C20:4	0	0.527 ± 0.048^{ABb}	0.677 ± 0.034^{ABa}	0.730 ± 0.050^{ABa}	0.01
	6	0.587 ± 0.049^{Ab}	0.677 ± 0.033^{Aa}	0.668 ± 0.033^{BDa}	0.001
	12	0.484 ± 0.077^{ABCb}	0.635 ± 0.037^{ABa}	0.651 ± 0.050^{CDa}	0.02
	18	0.413 ± 0.084^{BCb}	0.609 ± 0.109^{Aa}	0.684 ± 0.019^{ABCa}	0.01
	24	0.338 ± 0.025^{Cb}	0.656 ± 0.090^{Ba}	0.674 ± 0.027^{ACa}	0.001
	30	0.375 ± 0.028^{Cb}	0.701 ± 0.021^{ABa}	0.789 ± 0.036^{Aa}	0.001
Pr>F Ripening time		0.001	0.001	0.01	

^{a,b,c,d}Means followed by different lower case on the row, and different capital letters in columns are significantly different ($p < 0.05$) by Tukey's test.

Table 5. Continued...

Fatty acid, % of fat	Ripening time (days)	Treatment			Pr > F treatment
		Control	Rosemary	Rosemary + Celery	
Total omega-9	0	42.80 ± 1.38^{AB}	42.63 ± 1.24	43.19 ± 0.85	0.74
	6	42.31 ± 1.06^{B}	42.13 ± 1.30	42.06 ± 1.21	0.94
	12	41.21 ± 1.30^{AB}	41.78 ± 0.55	41.20 ± 1.45	0.67
	18	42.02 ± 1.90^{AB}	42.16 ± 1.37	42.19 ± 1.82	0.97
	24	40.70 ± 2.75^{AB}	40.32 ± 3.13	42.06 ± 1.22	0.18
	30	43.48 ± 0.98^{A}	42.41 ± 1.09	42.51 ± 1.15	0.29
Pr>F Ripening time		0.02	0.23	0.70	
Total omega-6	0	14.42 ± 1.29^{A}	15.02 ± 1.04^{B}	15.20 ± 1.10	0.13
	6	14.62 ± 0.90^{A}	14.96 ± 1.00^{B}	14.92 ± 1.01	0.08
	12	14.40 ± 1.05^{Bb}	15.69 ± 0.64^{ABa}	15.53 ± 0.52^{a}	0.001
	18	12.46 ± 0.48^{BD}	14.10 ± 1.00^{AB}	14.74 ± 0.90	0.17
	24	13.40 ± 0.08^{Dc}	15.81 ± 0.14^{Ab}	16.65 ± 0.46^{a}	0.001
	30	13.01 ± 0.80^{Bb}	14.82 ± 0.97^{ABa}	14.92 ± 0.95^{a}	0.001
Pr>F Ripening time		0.001	0.04	0.72	

^{a,b,c,d}Means followed by different lower case on the row, and different capital letters in columns are significantly different ($p < 0.05$) by Tukey's test.

and remained stable from that point on. The extracts had no significant effect ($p < 0.05$) on the a* values (redness) of the products. With regard to the ripening time, in the Control treatment, redness was higher ($p < 0.05$) at days 18 and 30 of ripening than at the other days.

Salami of the Rosemary and Rosemary + celery treatments had higher ($p < 0.001$) b* values at the 12th and 18th days of ripening. At the 24th day, no difference was detected between the treatments, and at the 30th day, Rosemary + celery treatment salami showed higher ($p < 0.001$) b* values than those of the other treatments.

It was observed an increase ($p < 0.01$) in the color saturation index during the ripening period in the Control treatment, but the other treatments showed no influence of ripening time. At the 24th day of ripening, the color saturation index was higher ($p < 0.04$) in the salami of the Rosemary treatment compared with the other treatments. Higher saturation index, as well as, higher a* values and lower b* values indicate a more favorable color, or less discoloration (fading) of the product. The highest L* values observed in the treatments containing smaller amounts of NO_3/NO_2 (Rosemary and Rosemary + Celery treatments) indicate lighter color. This difference disappeared up to day 18 of ripening suggesting that the salami with lower content of NO_3/NO_2 took longer to develop the typical color of the product, which results from the synthesis of nitrosyl myoglobin (FOX JUNIOR, 1987). These results are in agreement with those found by Sindelar et al. (2007a, b), who observed no changes in cured color in sausages and ham produced with addition of vegetable juice powder and without curing salt.

Since the treatments showed similar color saturation index for most of the periods evaluated, as well as similar a* values at all ripening times, despite the higher b* values, it can be considered that the products containing rosemary and rosemary + celery developed adequate color and resistance to discoloration, especially from day 18, when L* values (reflectance) became similar between the treatments. This is important from the point of view of consumer preferences since color is among the most appealing characteristics affecting the decision-making for buying meat (BREWER, 1998). Presence of antioxidants in the tissues can reduce the oxidation of muscle pigments, thus improving color during extended storage (ZANARDI et al., 1998).

3.3 Fatty acids

Fatty acids (C16:1, C18:1, and C20:2) and the sum of saturated fatty acids showed differences ($p < 0.05$) between the treatments at some ripening periods (Table 5). The sum of omega-9 fatty acids (C16:1 and C18:1) was not different ($p > 0.05$) between the treatments at any ripening time. On the other hand, C18:2 was lower ($p < 0.05$) in the Control treatment than in the other two treatments from the 6th day and nearly in all ripening times; the same behavior was found for the sum of omega-6 fatty acids (C18:2 and C20:2; $p < 0.01$) from day 12. The content of C20:4 fatty acid was lower ($p < 0.05$) in the Control treatment at all ripening times.

Higher levels of C20:4 from day zero in the treatments with rosemary extract could be the result of the presence of this fatty acid in the oil extract. The reduction of the content of C18:2, C20:4, and the sum of omega-6, throughout the ripening period in the Control treatment may have been caused by the degradation of fatty acids (Beta oxidation). In the treatments containing rosemary extract, the levels of these fatty acids did not change during the ripening period suggesting a protective effect of this product against polyunsaturated fatty acid oxidation, greater than that provided by NO_2.

These results are consistent with those of previous studies, in which the antioxidant effect of rosemary in foodstuffs has been demonstrated for chicken sausages (RIZNAR et al., 2006) and raw pork (SHAN et al., 2009). Basmacioglu, Tokusoglu and Ergul (2004) reported that rosemary oil can prevent lipid oxidation in chicken meat through their inclusion in the diet of these animals, even when enriched with omega-3 fatty acids.

Volatile compounds resultant from lipid oxidation during processing, ripening, and cooking are important components for taste and flavor development, and, consequently, for acceptability of meat and meat products by consumers. Polyunsaturated fatty acids have low oxidative stability, which may result in the production of undesirable volatile compounds, responsible by off-flavors, thus negatively influencing flavor and taste in meat and meat products (DAZA et al., 2005; MUSELLA et al., 2009). The compounds resulting from fat deterioration are also a potential risk to human health, with the production of toxic or toxigenic substances (MORRISSEY et al., 1998). Therefore, the importance of the protective effects of vegetable extracts against fatty acids oxidation is worth noting.

4 Conclusions

The production of salami containing rosemary extract and reduced levels of added nitrite and nitrate resulted in a product with physical and chemical characteristics and residual levels of nitrite and nitrate similar to those of conventional salami.

The addition of rosemary extract and celery powder in the formulation did not inhibit the development of the starter culture added to the salami confirming the influence of the cultures on important properties of the final product.

There is evidence that the rosemary extract reduced the oxidation of some types of unsaturated fatty acids during ripening. At longer ripening periods, the salamis with rosemary extract may have advantages in terms of fat oxidation, but this finding needs to be confirmed by further studies with direct evaluation of the oxidation products.

Reduced addition of NO_3 and NO_2 to the salami did not affect the levels of residual nitrate and nitrite, but it influenced the color of the product during the early stages of ripening. This difference gradually disappeared over the ripening period. Therefore, with longer ripening periods, products tend to be similar in quality.

The specific products used in the present study originated from dehydrated celery proved to be effective sources of NO_3 and NO_2 for color development and for maintenance of low residual levels of NO_3 and NO_2 compared with the same added levels of NO_3+NO_2 from curing salt. However, the excessive acidification of the product indicates the need to better evaluate its use in fermented salami.

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