

## Research Article

# Adding Blends of NaCl, KCl, and CaCl<sub>2</sub> to Low-Sodium Dry Fermented Sausages: Effects on Lipid Oxidation on Curing Process and Shelf Life

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The effect of a 50% reduction of NaCl and its replacement by KCl,  $CaCl_2$ , and a blend of KCl and  $CaCl_2$  (1:1) on lipid oxidation of dry fermented sausages was investigated. We found that a 50% reduction in NaCl decreased the intensity of the reactions to lipid oxidation, while treatments with added  $CaCl_2$  resulted in increased lipid oxidation during manufacture and storage. Fatty acid composition also changed owing to the presence of KCl and  $CaCl_2$ , showing a decrease in saturated, monounsaturated, and polyunsaturated fatty acids after 30 days of storage. Furthermore, a decreased intensity of  $L^*$  and increased  $b^*$  values were found in salamis with  $CaCl_2$ . These results suggest that using  $CaCl_2$  as a substitute for NaCl increases the intensity of oxidative reactions while the addition of KCl could be a good alternative to reduce the NaCl content in fermented meat products.

#### 1. Introduction

Lipolytic reactions result from the most important biochemical events occurring in the lipid fraction during the processing and storage of fermented meat products, directly influencing the overall quality of the final product [1]. These reactions are influenced by many factors, including the processing conditions, pH, presence of metals, and nature and contents of salt [2]. In particular, reactions involving lipid oxidation are important in the development of the aroma and flavor of salamis; however, they must be controlled to ensure that the excess and transformation of these compounds do not affect the desired characteristics or risks consumer health [3].

Sodium chloride [NaCl] is one of the main ingredients used in salami processing, playing an important role in technological, microbiological, and sensory quality. This ingredient, however, is the main source of sodium, an element targeted for reduction throughout the production chain of processed foods, especially meat products [4, 5].

Sodium chloride (NaCl) has been described by many authors as a prooxidant compound capable of influencing the development and intensity of lipid reactions in salamis. Its effect on lipid oxidation appears to be due either to the reactive action of chloride ions on lipids [6] or to the solubilization of iron by chloride ions, stimulating lipid peroxidation [7]. According to Toldrá et al. [8], NaCl content can also interfere with the activity of endogenous enzymes, thus changing the intensity of lipolytic reactions. In previous papers, our group investigated the volatile compounds, proteolysis, rheological, physicochemical, microbiological, and sensory properties of salamis that had their NaCl content reduced or replaced with KCl, CaCl<sub>2</sub>, or a blend of KCl and CaCl<sub>2</sub> [5, 9–11]. However, the effects of this reformulation on the attributes of lipid oxidation still require investigation. Hence, this study contributes to the body of knowledge on this topic by evaluating the oxidative stability of reduced NaCl salamis during the curing process and storage of salamis that had their NaCl content reduced or replaced with KCl, CaCl<sub>2</sub>, or a blend of KCl and CaCl<sub>2</sub>.

#### 2. Materials and Methods

2.1. Treatments and Processing of Salamis. Treatments with 50% reduction or replacement of NaCl by KCl, CaCl<sub>2</sub>, and a blend of KCl and CaCl<sub>2</sub> (1:1) were prepared as follows: control (2.5% NaCl), 50% salt reduced (1.25% NaCl, F1), 50% replaced by KCl (1.25% NaCl and 1.25% KCl, F2), 50% replaced by CaCl<sub>2</sub> (1.25% NaCl and 1.25% CaCl<sub>2</sub>, F3), and 50% replaced by KCl and CaCl<sub>2</sub> (1.25% NaCl, 0.625% KCl, and 0.625 CaCl<sub>2</sub>, F4). Processing was carried out in the Laboratory of Meat and Derivatives at the Faculty of Food Engineering, University of Campinas (São Paulo, Brazil). The manufacturing process was as described by Dos Santos et al. [5]. Salamis were produced with pork meat (650 g/kg), beef (200 g/kg), and pork back fat (150 g/kg) and were mixed with the correct amount of NaCl and other ingredients for each treatment. The following ingredients were added to the meat mixture in each treatment: glu- $\cos(0.5 \text{ g/kg})$ , sucrose (0.5 g/kg), sodium nitrite (0.15 g/kg), sodium nitrate (0.15 g/kg), white pepper (2 g/kg), nutmeg (0.02 g/kg), sodium ascorbate (0.25 g/kg), and a starter culture (0.25 g/kg; Bactoferm T-SPX Chr. Hansen) consisting of Pediococcus pentosaceus and Staphylococcus xylosus. After complete homogenization, the batter of each treatment was packaged and taken to the salami manufacturing chamber. For each treatment, 90 sausages were produced of approximately 300 g each. The parameters for temperature and relative humidity were as follows: 1st day (25°C/95%), 2nd day (24°C/93%), 3rd day (23°C/90%), 4th day (22°C/85%), 5th day ( $21^{\circ}C/80^{\circ}$ ), 6th day ( $20^{\circ}C/75^{\circ}$ ), and from 7th day until the end of maturation, 19 days total (18°C/75%). Air velocity was maintained below 5 m/s. At the end of the manufacturing process (19 days or time point 0), salamis were vacuumpacked (Unipac/Univac B320-Minivac CU18, Selovac, São Paulo, SP, Brazil) and stored at 25  $\pm$  1°C for 90 days.

2.2. Lipid Oxidation and Fatty Acid Composition. Lipid oxidation and fatty acid composition were analyzed at the beginning of processing (day 0), at the end of fermentation (day 7), at the end of processing (day 19), and after 30, 60, and 90 days of storage at 25°C. For each time point, three pieces of each treatment were collected and immediately frozen at  $-80^{\circ}$ C until analysis.

The lipid oxidation of salamis was measured by the amount of thiobarbituric acid-reactive substances (TBARS), as described by Bruna et al. [12], using trichloroacetic acid instead of perchloric acid as the solvent. The results were expressed in  $\mu$ g of malondialdehyde (MDA)/g for each sample.

Lipids were extracted using chloroform/methanol/water (1:2:0.6 V/V) as described by Bligh and Dyer [13], and they underwent transesterification using the method of Hartman and Lago [14]. Fatty acid methyl esters (FAMEs) were analyzed in a gas chromatograph equipped with a flame ionization detector (GC-FID), Varian Star 3400CX (CA, USA), by using an autosampler model 4200 (CA, USA). A  $1\mu$ L injection of the sample was performed using a split type/splitless injector operating in split mode 1:50 at 250°C. Hydrogen was used as a carrier gas at a constant pressure

of 30 psi and an initial flow rate of 0.8 mL/min. FAMEs were separated into capillary columns (SP-2560 Supelco, USA; 100 m × 0.25 mm × 0.20  $\mu$ m). The initial column heating temperature was 80°C, which was maintained for 30 s, followed by an increase of 15°C/min until 175°C was reached, followed by a second increase of 0.5°C/min until 190°C and a final increase of 8°C/min until 240°C. The column was then kept isothermal for 15 mins. The detector temperature was maintained at 240°C.

FAMEs were identified by comparing the retention times of the analytes with authentic standards FAME Mix-37, P/N 47885-U; methyl ester 11-trans-vaccenic acid, P/N 46905-U, mixed isomers of conjugated linoleic acid methyl esters, P/N 05632, and mixed isomers of linoleic and linolenic acid methyl esters, P/N 47791; and cis-7,10,13,16,19docosapentaenoic acid methyl ester, P/N 47563-U, all produced by Supelco (PA, USA) and purchased from Sigma-Aldrich. Quantification was carried out using the internal standard methyl tricosanoate (C23:0), and the quantitative method was validated by Visentainer [15]. The results were expressed in mg/g lipid.

2.3. Instrumental Color. Color was determined at the end of the curing process and storage using a spectrophotometercolorimeter (model CM-5; Konica Minolta) with spectral reflectance included as a calibration mode, Standard Illuminant D65, and an observation angle of 10°, operating under the CIE system ( $L^*a^*b^*$ ). The values of  $L^*$  (luminosity),  $a^*$  (intensity of red), and  $b^*$  (intensity of yellow) were determined. Whiteness value was calculated using the following formula [16]:

Whiteness = 
$$100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$
, (1)

where  $L^*$  = lightness on a 0–100 scale from black to white,  $a^*$  = scale of red (+) or green (–), and  $b^*$  = scale of yellow (+) or blue (–).

2.4. Statistical Analysis. In each manufacture, three sample units (salamis) were taken per sampling day (n = 9). All analyses were performed in triplicate. The results reported in this study are the mean obtained from all the data recorded for each parameter analyzed. Data were evaluated by analysis of variance (one-way ANOVA) and Fisher's test (P < 0.05) to identify the differences among salamis assessed during the curing process and over their shelf life. Statistica V.8 software was used for data analysis.

#### 3. Results and Discussion

3.1. Effects of Salts on Lipid Oxidation. Lipid oxidation is one of the main phenomena responsible for the reduced shelf life and sensory quality of fermented meat products. Malondialdehyde (MDA) is a typical product of lipid oxidation, formed mainly by the degradation of polyunsaturated fatty acids [17]. Table 1 shows the effect on lipolytic reactions (TBARS) of reducing or replacing NaCl with KCl and/or CaCl<sub>2</sub> during the manufacture and storage of salamis.

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TABLE 1: Malondialdehyde content (µg MDA/g sample) during curing process and storage of salamis with NaCl reduced or replaced by KCl
and/or CaCl <sub>2</sub> .

Treatments		Time (days)							
	$0^{\mathrm{a}}$	7	19/0 <sup>b</sup>	30	60	90	SEM*		
Control	$0.07^{\mathrm{aD}}$	0.126 <sup>bC</sup>	0.144 <sup>bcBC</sup>	$0.170^{bB}$	0.181 <sup>cB</sup>	0.337 <sup>aA</sup>	0.01		
F1	$0.09^{\mathrm{aD}}$	$0.124^{bCD}$	0.131 <sup>cC</sup>	$0.150^{cB}$	$0.156^{dB}$	0.265 <sup>bA</sup>	0.01		
F2	$0.08^{\mathrm{aC}}$	0.133 <sup>bB</sup>	0.165 <sup>abB</sup>	$0.178^{\mathrm{bB}}$	$0.187^{cB}$	0.365 <sup>aA</sup>	0.01		
F3	$0.09^{aE}$	$0.174^{aD}$	$0.188^{aD}$	0.286 <sup>aC</sup>	0.331 <sup>aB</sup>	0.388 <sup>aA</sup>	0.02		
F4	$0.09^{aE}$	$0.151^{abD}$	0.186 <sup>aC</sup>	0.253 <sup>aB</sup>	0.263 <sup>bB</sup>	0.395 <sup>aA</sup>	0.02		

<sup>a</sup>Days of curing process (0, 7, and 19).

<sup>b</sup>Days of storage (0, 30, 60, and 90).

\*Standard error of the means (n = 9).

Different small letters in the same column indicate significant differences at P < 0.05 (Fisher's test). Different capital letters in each row for each parameter indicate significant differences at P < 0.05 (Fisher's test). Control, 100% NaCl; F1, 50% NaCl; F2, 50% NaCl and 50% KCl; F3, 50% NaCl and 50% CaCl<sub>2</sub>; F4, 50% NaCl, 25% KCl, and 25% CaCl<sub>2</sub>.

At the beginning of manufacture (0 days), the measured TBARS amounts were not significantly different among the treatments. On the 7th day of manufacture, only treatment F3 (50% NaCl and 50% CaCl<sub>2</sub>) presented TBARS values higher than the control (P < 0.05). At the end of the curing process (19/0 d), the treatments with the addition of 50% (F3) and 25% CaCl<sub>2</sub> (F4) showed significantly higher amounts of TBARS compared to the control group. This behavior continued until 60 days of storage. At the final time point of 90 days, only salamis produced with a 50% reduction of NaCl (F1) differed from the control group, with lower TBARS amounts. The data just reported agree with previous measurements of TBARS in dry fermented meat products [18, 19].

There are many postulations as to how sodium chloride acts as a prooxidant. Kanner and Rosenthal [20] argue that NaCl acts as a prooxidant by displacing the iron ions with sodium in the heme pigments of the muscle tissue, whereas others recognize the chloride ion acting upon the lipid as the source [21]. According to Hernández et al. [22], lipid oxidation is enhanced by increasing ionic strength. This finding can partly explain the greater lipid oxidation observed in treatments with CaCl<sub>2</sub>, since the ionic strength of divalent salts is higher than that of monovalent salts. This outcome is in agreement with those reported by dos Santos et al. [9] who reported that the addition of CaCl<sub>2</sub> increased the generation of hexanal and (E)-hept-2-enal and other volatiles from lipid oxidation during processing and storage of lowsodium fermented sausages. An increase in lipid oxidation with the addition of divalent salts was reported by Zanardi et al. [23], who replace NaCl with a blend of salts (KCl, CaCl<sub>2</sub>, and MgCl<sub>2</sub>) in Italian-type salamis. High TBARS levels were also found by Flores et al. [24] when using 0.5% CaCl<sub>2</sub> in salamis.

According to Rhee and Ziprin [25], the addition of 0.5–2.5% NaCl in meat products is enough to provide a prooxidant effect, thus increasing oxidative reactions during the processing and storage stages of products. In their study, salamis produced with 50% reduction of NaCl (F1 - 1.25%) had lower TBARS values ( $P \le 0.05$ ) than the control group.

In the evaluation of TBARS levels during the 90-day storage period, a progressive increase was observed in lipid

oxidation across all treatments. This same behavior has been reported by other researchers studying the development of lipid oxidation in salamis [26]. Increasing lipid oxidation can adversely affect the sensory quality of dry fermented sausages by generating compounds of degradation, such as nalkenals and dienals [27]. Additionally, oxidation can affect the nutritional value of products by decomposing vitamins and unsaturated fatty acids [28].

3.2. Composition of Fatty Acids. Fatty acid (FA) content (Tables 2-4) was tracked during the manufacture and storage of reduced NaCl salamis. The composition of FA is one of the most important components that can change during processing, affecting sensory proprieties and nutritional value of food [29]. FA composition may be directly affected by lipid-oxidation reactions, and unsaturated fatty acids are the most susceptible to these changes [30]. In general, we observed that the content of saturated fatty acids (SFA) (Table 2), monounsaturated fatty acids (MUFA) (Table 3), and polyunsaturated fatty acids (PUFA) (Table 4) decreased after 30 days of storage, especially in the treatments where NaCl was reduced and replaced by KCl (F2), CaCl<sub>2</sub> (F3), and a blend of KCl and CaCl<sub>2</sub> (F4). The nutritional recommendations are that the PUFA/SFA ratio should be above 0.45 [29]. The PUFA/SFA ratios in this study were closer to the values for the human diet (Table 4). The PUFA/SFA ratios decreased significantly during the curing process and storage in samples F2, F3, and F4. These results suggest that higher lipolytic activity may be occurring in salamis where NaCl content has been replaced by other chloride salts.

According to Coutron-Gambotti et al. [27], during the processing of fermented products, lipid oxidation leads to decreased unsaturated, long-chain fatty acids and the increased generation of free fatty acids and phospholipids. For instance, Quintanilla et al. [2] found higher free fatty acid levels in dry fermented sausages produced with 1.5% NaCl and 1% KCl, compared with samples containing 3% NaCl. In our study, the reduction of unsaturated fatty acids and the high levels of TBARS observed mainly in the treatments containing  $CaCl_2$  suggest the high lipolytic activity of these salts during the storage of salamis. This outcome is in

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TABLE 2: Content of saturated fatty acids (SFA) in the lipid fraction (mg/g fat) during curing process and storage of salamis with NaCl reduced
or replaced by KCl and/or CaCl <sub>2</sub> .

		C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	SFA
	Control	12.0	2.1 <sup>A</sup>	215.7 <sup>b</sup>	4.1 <sup>AB</sup>	121.3	2.3 <sup>bA</sup>	357.6 <sup>AB</sup>
	F1	11.9	$1.8^{\text{A}}$	237.9 <sup>abA</sup>	3.5	119.2	2.3 <sup>bA</sup>	376.6 <sup>A</sup>
0 d <sup>a</sup>	F2	12.7	$1.7^{AB}$	257.3 <sup>aA</sup>	3.8 <sup>AB</sup>	127.0 <sup>AB</sup>	$2.9^{\mathrm{aA}}$	405.2 <sup>A</sup>
	F3	11.8 <sup>A</sup>	2.1 <sup>A</sup>	222.3 <sup>bABC</sup>	3.8 <sup>AB</sup>	110.2 <sup>ABC</sup>	2.2 <sup>bA</sup>	352.4 <sup>ABC</sup>
	F4	11.2 <sup>AB</sup>	$1.7^{\text{A}}$	217.3 <sup>bBC</sup>	$4.0^{AB}$	110.3 <sup>B</sup>	2.3 <sup>bAB</sup>	346.6 <sup>BC</sup>
	Control	12.5	2.1 <sup>A</sup>	246.7	4.2 <sup>AB</sup>	119.9	2.1 <sup>AB</sup>	387.5 <sup>A</sup>
	F1	11.9	$1.8^{\text{A}}$	242.4 <sup>A</sup>	4.0	122.5	2.3 <sup>A</sup>	384.9 <sup>A</sup>
7 d	F2	13.0	$2.0^{\text{A}}$	258.3 <sup>A</sup>	$4.1^{AB}$	131.4 <sup>A</sup>	$2.0^{B}$	$410.8^{A}$
	F3	12.0 <sup>A</sup>	$1.7^{AB}$	238.6 <sup>AB</sup>	4.3 <sup>A</sup>	122.6 <sup>AB</sup>	2.2 <sup>A</sup>	$381.4^{AB}$
	F4	13.0 <sup>AB</sup>	1.9 <sup>A</sup>	$254.7^{AB}$	$4.4^{\mathrm{A}}$	127.9 <sup>AB</sup>	2.2 <sup>AB</sup>	$404.1^{AB}$
19/0 d <sup>b</sup>	Control	12.5 <sup>ab</sup>	$1.8^{abA}$	245.6 <sup>ab</sup>	4.4 <sup>A</sup>	125.1 <sup>b</sup>	2.0 <sup>AB</sup>	391.4 <sup>A</sup>
	F1	12.0 <sup>bc</sup>	$1.8^{abA}$	241.7 <sup>bA</sup>	4.2	122.4 <sup>b</sup>	2.2 <sup>A</sup>	384.3 <sup>A</sup>
	F2	10.3 <sup>c</sup>	$1.4^{\mathrm{bAB}}$	209.7 <sup>bB</sup>	3.9 <sup>AB</sup>	105.8 <sup>cC</sup>	2.1 <sup>B</sup>	333.2 <sup>BC</sup>
	F3	12.6 <sup>abA</sup>	$1.6^{abB}$	247.0 <sup>abA</sup>	4.5 <sup>A</sup>	125.8 <sup>bA</sup>	$2.4^{\mathrm{A}}$	393.9 <sup>A</sup>
	F4	$14.1^{aA}$	2.1 <sup>aA</sup>	284.9 <sup>aAB</sup>	4.7 <sup>A</sup>	144.8 <sup>aA</sup>	$2.4^{\mathrm{A}}$	453.0 <sup>A</sup>
	Control	10.3 <sup>a</sup>	1.0 <sup>B</sup>	194.4 <sup>ab</sup>	3.3 <sup>B</sup>	107.9 <sup>ab</sup>	2.1 <sup>AB</sup>	335.6 <sup>B</sup>
	F1	9.0 <sup>b</sup>	$1.0^{\mathrm{B}}$	152.6 <sup>bB</sup>	3.2	116.9 <sup>a</sup>	$1.9^{AB}$	300.8 <sup>B</sup>
30 d	F2	10.5 <sup>a</sup>	$0.8^{ m C}$	208.3 <sup>aB</sup>	3.3 <sup>B</sup>	$104.0^{abC}$	$2.0^{B}$	329.0 <sup>C</sup>
	F3	$10.6^{aB}$	$1.1^{B}$	$189.5^{\mathrm{abC}}$	$3.5^{B}$	94.1 <sup>abC</sup>	$1.7^{\mathrm{B}}$	299.5 <sup>C</sup>
	F4	9.0 <sup>abB</sup>	$1.1^{B}$	$181.0^{\mathrm{abC}}$	3.1 <sup>B</sup>	89.9 <sup>bB</sup>	$1.5^{\circ}$	285.5 <sup>C</sup>
	Control	11.0	0.6 <sup>bB</sup>	228.9 <sup>ab</sup>	3.4 <sup>AB</sup>	113.2	1.5 <sup>bB</sup>	359.0 <sup>AB</sup>
	F1	10.8	0.6 <sup>bBC</sup>	180.5 <sup>cB</sup>	3.4	113.0	$1.4^{bB}$	328.4 <sup>AB</sup>
60 d	F2	10.4	$0.7^{bC}$	207.6 <sup>bcB</sup>	3.3 <sup>B</sup>	106.7 <sup>BC</sup>	$1.1^{bB}$	329.8 <sup>C</sup>
	F3	$11.8^{B}$	0.6 <sup>bC</sup>	233.7 <sup>abAB</sup>	3.8 <sup>AB</sup>	$118.1^{AB}$	1.5 <sup>bB</sup>	369.5 <sup>AB</sup>
	F4	13.0 <sup>AB</sup>	$0.9^{aBC}$	262.3 <sup>aAB</sup>	4.2 <sup>A</sup>	130.0 <sup>AB</sup>	$1.6^{aB}$	412.8 <sup>AB</sup>
	Control	12.9	$0.4^{\mathrm{B}}$	240.3	3.6 <sup>AB</sup>	110.0	0.9 <sup>C</sup>	368.1 <sup>AB</sup>
	F1	11.8	0.3 <sup>C</sup>	236.4 <sup>A</sup>	3.6	112.1	$1.1^{B}$	365.3 <sup>A</sup>
90 d	F2	12.1	$0.5^{ m C}$	$258.7^{AB}$	4.3 <sup>A</sup>	124.1 <sup>ABC</sup>	$2.0^{B}$	381.6 <sup>AB</sup>
	F3	$10.7^{\mathrm{B}}$	$0.4^{ m D}$	210.3 <sup>BC</sup>	3.4 <sup>B</sup>	105.2 <sup>BC</sup>	$1.8^{\mathrm{B}}$	331.8 <sup>BC</sup>
	F4	12.2 <sup>B</sup>	$0.5^{ m C}$	234.8 <sup>AB</sup>	4.2 <sup>A</sup>	114.5 <sup>B</sup>	1.6 <sup>BC</sup>	367.8 <sup>B</sup>
	SEM*	0.4	0.1	0.2	5.8	0.5	0.2	8.3

<sup>a</sup>Days of curing process (0, 7, and 19).

<sup>b</sup>Days of storage (0, 30, 60, and 90).

\*Standard error of the means (n = 9).

Different small letters in the same column indicate significant differences at P < 0.05 (Fisher's test) between treatments. Different capital letters in each column for each parameter indicate significant differences at P < 0.05 (Fisher's test) along the manufacturing and storage. Control, 100% NaCl; F1, 50% NaCl; F2, 50% NaCl and 50% KCl; F3, 50% NaCl and 50% CaCl<sub>2</sub>; F4, 50% NaCl, 25% KCl, and 25% CaCl<sub>2</sub>.

agreement with the evolution of TBARS levels during the manufacture and storage of salamis (Table 1).

The fatty acids predominately found in salamis were palmitic (C16:0), stearic (C18:0), oleic (C18:1(n-9)), and linoleic (C18:2(n-6)). These fatty acids are commonly found at high concentrations in meat products [31]. At each time point, some differences between the control group and treatments were observed. These differences can be attributed to high heterogeneity, which is typical in salamis [32], since the processing conditions and raw meat material used were the same for all the treatments.

3.3. Effects of Salts on Color. The color of salamis is one of the sensory properties influenced by the reduction or

replacement of NaCl [32]. In this study, differences in the color parameters  $L^*$ ,  $a^*$ , and  $b^*$  as well as whiteness were observed in the modified salamis compared with the control group (Table 5). During storage, F1 and F2 showed no difference for the  $L^*$  value. However, the  $L^*$  values of salamis in F3 and F4 decreased over time, differing from the control group both for the final product (19/0 days) and at the end of their storage (90 days) (P < 0.05). Aliño et al. [33] found lower  $L^*$  values for cured loins with 20% CaCl<sub>2</sub> and 10% MgCl<sub>2</sub> added. From 60 days of storage, salamis produced with 50% NaCl (F1) had a higher  $L^*$  value compared with the control group; this higher value was maintained until the end of storage.

TABLE 3: Content of monounsaturated fatty acids (MUFA) in the lipid fraction (mg/g fat) during curing process and storage of salamis with NaCl reduced or replaced by KCl and/or CaCl<sub>2</sub>.

		C14:1	C16:1	C17:1	C18:1 9t	C18:1(n-9)	C20:1(n-9)	MUFA
	С	$0.7^{AB}$	16.9 <sup>AB</sup>	2.7 <sup>AB</sup>	1.3 <sup>ab</sup>	367.4 <sup>abA</sup>	7.4 <sup>abAB</sup>	396.5 <sup>A</sup>
	F1	0.8	15.1	2.6	0.8 <sup>c</sup>	340.2 <sup>abA</sup>	7.3 <sup>ab</sup>	366.8 <sup>A</sup>
0 d <sup>a</sup>	F2	$0.9^{\mathrm{A}}$	$14.7^{AB}$	$2.5^{BC}$	$1.4^{a}$	377.9 <sup>aA</sup>	8.3 <sup>aA</sup>	405.6 <sup>AB</sup>
	F3	0.8	16.3 <sup>A</sup>	$2.4^{BC}$	$1.2^{ab}$	329.6 <sup>bAB</sup>	6.5 <sup>bBCD</sup>	356.7 <sup>BC</sup>
	F4	$0.8^{\mathrm{BC}}$	15.1 <sup>AB</sup>	2.5 <sup>AB</sup>	1.0 <sup>bc</sup>	334.6 <sup>abB</sup>	7.2 <sup>ab</sup>	361.1 <sup>BC</sup>
	С	0.8 <sup>A</sup>	18.3 <sup>A</sup>	3.0 <sup>A</sup>	1.1	372.1 <sup>A</sup>	7.7 <sup>A</sup>	403.0 <sup>A</sup>
	F1	0.8	16.7	2.7	0.8	372.3 <sup>A</sup>	7.6	401.2 <sup>A</sup>
7 d	F2	0.9 <sup>A</sup>	17.0 <sup>A</sup>	3.4 <sup>A</sup>	1.3	396.6 <sup>A</sup>	$7.7^{AB}$	427.1 <sup>A</sup>
	F3	0.8	16.7 <sup>A</sup>	3.0 <sup>A</sup>	1.2	359.0 <sup>A</sup>	$7.4^{AB}$	388.1 <sup>AB</sup>
	F4	$0.8^{AB}$	17.0 <sup>A</sup>	2.9 <sup>A</sup>	1.1	372.1 <sup>B</sup>	8.1	402.0 <sup>B</sup>
	С	0.9 <sup>A</sup>	17.4 <sup>aAB</sup>	2.9 <sup>aA</sup>	0.8	372.5 <sup>bA</sup>	7.8 <sup>A</sup>	402.3 <sup>A</sup>
	F1	0.8	16.9 <sup>ab</sup>	2.8 <sup>ab</sup>	0.9	369.3 <sup>bcA</sup>	7.3	398.0 <sup>A</sup>
19/0 d <sup>b</sup>	F2	$0.7^{AB}$	$14.1^{bAB}$	2.3 <sup>bBC</sup>	1.0	322.4 <sup>cAB</sup>	6.7 <sup>BC</sup>	347.2 <sup>BC</sup>
	F3	0.9	16.9 <sup>abA</sup>	$2.7^{abAB}$	1.6	376.0 <sup>bA</sup>	8.0 <sup>A</sup>	406.1 <sup>A</sup>
	F4	$1.0^{\mathrm{A}}$	18.5 <sup>aA</sup>	3.0 <sup>aA</sup>	1.0	435.4 <sup>aA</sup>	6.2	465.1 <sup>A</sup>
	С	0.6 <sup>B</sup>	13.3 <sup>abC</sup>	2.1 <sup>B</sup>	1.3	299.1 <sup>abAB</sup>	6.3 <sup>BC</sup>	337.4 <sup>B</sup>
	F1	0.6	15.8 <sup>a</sup>	2.1	1.4	348.5 <sup>aA</sup>	6.0	374.4 <sup>A</sup>
30 d	F2	$0.4^{\mathrm{B}}$	$14.4^{abAB}$	$2.4^{BC}$	1.2	$320.4^{abAB}$	6.4 <sup>BC</sup>	345.2 <sup>BC</sup>
	F3	0.7	13.0 <sup>abB</sup>	2.1 <sup>C</sup>	0.9	279.6 <sup>bB</sup>	$5.4^{\text{CD}}$	301.8 <sup>C</sup>
	F4	0.6 <sup>C</sup>	12.6 <sup>bB</sup>	$2.1^{B}$	1.0	268.1 <sup>bC</sup>	5.0	$289.4^{\mathrm{D}}$
	С	$0.8^{AB}$	15.3 <sup>BC</sup>	$2.1^{abB}$	1.6	342.9 <sup>abAB</sup>	6.7 <sup>ABC</sup>	369.4 <sup>AB</sup>
	F1	0.7	14.4	2.3 <sup>ab</sup>	1.4	264.5 <sup>cB</sup>	6.0	287.9 <sup>B</sup>
60 d	F2	$0.7^{AB}$	13.5 <sup>B</sup>	$2.2^{abBC}$	0.8	307.2 <sup>bcB</sup>	5.4 <sup>C</sup>	329.8 <sup>C</sup>
	F3	0.7	15.3 <sup>B</sup>	1.8 <sup>bC</sup>	1.3	330.7 <sup>abB</sup>	$7.1^{ABC}$	356.9 <sup>BC</sup>
	F4	$0.8^{AB}$	17.0 <sup>A</sup>	$2.7^{aB}$	1.9	379.8 <sup>aC</sup>	7.8	$410.0^{AB}$
	С	0.8 <sup>A</sup>	15.2 <sup>BC</sup>	2.1 <sup>B</sup>	1.6	332.5 <sup>AB</sup>	5.4 <sup>C</sup>	357.6 <sup>AB</sup>
	F1	0.7	15.4	2.5	1.4	328.0 <sup>A</sup>	5.7	353.7 <sup>AB</sup>
90 d	F2	0.8 <sup>A</sup>	17.0 <sup>A</sup>	$2.8^{\mathrm{B}}$	1.7	373.2 <sup>A</sup>	$7.4^{AB}$	402.9 <sup>AB</sup>
	F3	0.7	$14.4^{AB}$	2.3 <sup>BC</sup>	0.9	308.2 <sup>B</sup>	$6.0^{\text{CD}}$	332.5 <sup>BC</sup>
	F4	$0.8^{AB}$	15.8 <sup>AB</sup>	2.7 <sup>A</sup>	1.3	315.0 <sup>C</sup>	7.0	342.6 <sup>CD</sup>
	SEM*	0.1	3.0	0.1	8.5	3.4	0.1	9.3

<sup>a</sup>Days of curing process (0, 7, and 19).

<sup>b</sup>Days of storage (0, 30, 60, and 90).

\*Standard error of the means (n = 9).

Different small letters in the same column indicate significant differences at P < 0.05 (Fisher's test) between treatments. Different capital letters in each column for each parameter indicate significant differences at P < 0.05 (Fisher's test) along the manufacturing and storage. Control (C), 100% NaCl; F1, 50% NaCl; F2, 50% NaCl and 50% KCl; F3, 50% NaCl and 50% CaCl<sub>2</sub>; F4, 50% NaCl, 25% KCl, and 25% CaCl<sub>2</sub>.

Salamis showed no differences in  $a^*$  values for the final product (19/0 d); however, reformulated salamis had higher  $a^*$  values from the time point of 60 days of storage, differing significantly from the control group. This finding can be explained by the negative effect of NaCl on the color of cured meat products during storage. According to Sakata and Nagata [34], NaCl interferes with heme pigment content; the higher the NaCl content, the lower the heme pigment content and, consequently, the lower the red intensity.

Yellow ( $b^*$ ) is one of the color parameters possibly related to lipid oxidation in salamis. In this study, the replacement of NaCl interfered with the intensity of the yellow color in salamis during storage for F3 (50% NaCl and 50% CaCl<sub>2</sub>) and F4 (50% NaCl, 25% KCl, and 25% CaCl<sub>2</sub>). These treatments displayed an increase in yellow intensity after 90 days of storage. This result suggests that the higher lipid oxidation observed in these treatments may have adversely interfered with the development of product color [35]. The comparison at each analysis time point between the control and reformulated salamis allowed us to observe higher  $b^*$  values (P < 0.05) for F1, F3, and F4 salamis at 19/0, 30, and 60 days. At the end of storage, only salamis F3 and F4 differed significantly from the control group in terms of  $b^*$  values. Gimeno et al. [36] observed higher  $b^*$  values in salamis with reduced NaCl upon the addition of a blend of chloride salts (NaCl, 10 g/kg; KCl, 5.52 g/kg; CaCl<sub>2</sub>, 7.38 g/kg).

A reduction in whiteness was observed during storage for salamis with added CaCl<sub>2</sub> (F3 and F4). This result is

TABLE 4: Content of polyunsaturated fatty acids (PUFA) in the lipid fraction (mg/g fat) during curing process and storage of salamis with NaCl reduced or replaced by KCl and/or CaCl<sub>2</sub>.

		C18:2(n-6)	C18:3(n-3)	C20:2	C20:3(n-6)	C20:4(n-6)	PUFA	PUFA/SFA
	С	139.4 <sup>bA</sup>	6.3 <sup>AB</sup>	5.5 <sup>bA</sup>	1.0	4.0 <sup>A</sup>	156.2	0.41 <sup>A</sup>
	F1	140.7 <sup>b</sup>	7.3 <sup>A</sup>	6.0 <sup>ab</sup>	1.1	4.0	158.6 <sup>A</sup>	$0.42^{A}$
$0 d^{a}$	F2	165.8 <sup>aA</sup>	8.0 <sup>A</sup>	6.8 <sup>aA</sup>	1.3 <sup>A</sup>	3.4 <sup>B</sup>	$185.4^{\text{A}}$	0.46 <sup>A</sup>
	F3	133.3 <sup>bB</sup>	7.8 <sup>A</sup>	$5.4^{bBC}$	$1.1^{BC}$	3.7 <sup>A</sup>	151.3 <sup>AB</sup>	0.43 <sup>A</sup>
	F4	145.0 <sup>bA</sup>	7.3 <sup>A</sup>	6.1 <sup>abA</sup>	$1.2^{ABC}$	3.7 <sup>AB</sup>	163.5 <sup>B</sup>	$0.47^{\mathrm{A}}$
	С	139.9 <sup>bA</sup>	7.1 <sup>A</sup>	6.0 <sup>A</sup>	1.4	3.9 <sup>AB</sup>	158.3	0.41 <sup>A</sup>
	F1	146.5 <sup>ab</sup>	7.1 <sup>AB</sup>	6.2	1.1	3.4	164.3 <sup>A</sup>	0.43 <sup>A</sup>
7 d	F2	161.9 <sup>aA</sup>	8.2 <sup>A</sup>	$7.0^{AB}$	1.3 <sup>A</sup>	$4.1^{A}$	$181.5^{A}$	$0.44^{\mathrm{A}}$
	F3	146.5 <sup>abA</sup>	$7.4^{\mathrm{A}}$	6.1 <sup>AB</sup>	$1.2^{B}$	3.5 <sup>A</sup>	$164.7^{A}$	$0.45^{A}$
	F4	158.9 <sup>aA</sup>	8.0 <sup>A</sup>	6.8 <sup>A</sup>	$1.0^{\mathrm{BC}}$	3.9 <sup>A</sup>	$178.6^{AB}$	$0.44^{\mathrm{A}}$
	С	147.0 <sup>bA</sup>	7.0 <sup>abA</sup>	6.1 <sup>bcA</sup>	$1.0^{ab}$	2.2 <sup>bBC</sup>	163.3	$0.42^{A}$
	F1	150.5 <sup>b</sup>	6.9 <sup>bAB</sup>	5.8 <sup>c</sup>	$1.1^{ab}$	3.5 <sup>ab</sup>	163.7 <sup>A</sup>	0.43 <sup>A</sup>
$19/0  d^b$	F2	145.6 <sup>bAB</sup>	6.7 <sup>bB</sup>	5.7 <sup>cA</sup>	$1.1^{abA}$	3.0 <sup>abBC</sup>	158.6 <sup>AB</sup>	$0.45^{A}$
	F3	150.9 <sup>bA</sup>	$7.5^{abA}$	6.5 <sup>abA</sup>	$0.8^{\mathrm{bC}}$	$3.4^{abA}$	167.7 <sup>A</sup>	$0.42^{B}$
	F4	188.7 <sup>aA</sup>	8.5 <sup>aA</sup>	7.2 <sup>aA</sup>	$1.3^{aABC}$	$4.0^{\mathrm{aA}}$	$202.7^{A}$	$0.45^{B}$
	С	121.8 <sup>AB</sup>	5.8 <sup>AB</sup>	5.0 <sup>AB</sup>	1.0	2.1 <sup>C</sup>	135.7	$0.40^{A}$
	F1	111.7	5.3 <sup>AB</sup>	$4.6^{B}$	1.1	2.1	124.9 <sup>AB</sup>	$0.42^{\mathrm{A}}$
30 d	F2	$128.0^{B}$	6.1 <sup>BC</sup>	5.0 <sup>C</sup>	$0.6^{\mathrm{B}}$	2.8 <sup>C</sup>	142.5 <sup>B</sup>	0.43 <sup>A</sup>
	F3	112.7 <sup>C</sup>	5.3 <sup>AB</sup>	$4.4^{\circ}$	$1.0^{\mathrm{BC}}$	$2.2^{B}$	125.6 <sup>C</sup>	$0.42^{B}$
	F4	106.6 <sup>B</sup>	$4.6^{\mathrm{B}}$	3.7 <sup>B</sup>	$0.9^{\rm C}$	2.9 <sup>C</sup>	118.7 <sup>C</sup>	$0.42^{\circ}$
	С	125.8 <sup>abB</sup>	6.1 <sup>AB</sup>	$4.4^{B}$	1.1 <sup>b</sup>	$2.4^{\mathrm{abC}}$	139.8	$0.40^{A}$
	F1	102.8 <sup>c</sup>	5.4 <sup>AB</sup>	4.9	$1.1^{b}$	2.5 <sup>ab</sup>	116.8 <sup>B</sup>	$0.40^{A}$
60 d	F2	129.2 <sup>bB</sup>	5.1 <sup>C</sup>	4.7 <sup>C</sup>	$1.2^{bA}$	$2.0^{bD}$	$142.2^{B}$	0.43 <sup>A</sup>
	F3	141.8 <sup>abC</sup>	6.0 <sup>AB</sup>	5.3 <sup>C</sup>	$1.8^{\mathrm{aA}}$	2.3 <sup>bB</sup>	157.2 <sup>AB</sup>	$0.41^{B}$
	F4	161.7 <sup>aA</sup>	7.0 <sup>A</sup>	6.1 <sup>A</sup>	$1.7^{aAB}$	3.6 <sup>aABC</sup>	$180.1^{AB}$	$0.40^{ m D}$
	С	$140.0^{AB}$	5.6 <sup>abcB</sup>	$4.4^{bB}$	1.3	2.8 <sup>BC</sup>	153.8	$0.40^{A}$
	F1	126.3	5.0 <sup>bcB</sup>	4.6 <sup>b</sup>	1.1	2.9	139.9 <sup>AB</sup>	0.39 <sup>A</sup>
90 d	F2	161.4 <sup>A</sup>	7.8 <sup>aA</sup>	6.8 <sup>aA</sup>	$1.4^{A}$	3.2 <sup>BC</sup>	180.6 <sup>A</sup>	$0.37^{B}$
	F3	126.0 <sup>BC</sup>	$4.0^{\text{cB}}$	$5.1^{abC}$	$1.0^{\mathrm{BC}}$	2.8 <sup>AB</sup>	139.0 <sup>BC</sup>	$0.42^{B}$
	F4	154.9 <sup>A</sup>	7.5 <sup>abA</sup>	7.0 <sup>aA</sup>	$1.4^{\mathrm{AB}}$	3.0 <sup>BC</sup>	173.8 <sup>AB</sup>	$0.41^{\mathrm{D}}$
	SEM*	0.3	0.2	0.2	0.1	0.3	3.7	0.01

<sup>a</sup>Days of curing process (0, 7, and 19).

<sup>b</sup>Days of storage (0, 30, 60, and 90).

\* Standard error of the means (n = 9).

Different small letters in the same column indicate significant differences at P < 0.05 (Fisher's test) between treatments. Different capital letters in each column for each parameter indicate significant differences at P < 0.05 (Fisher's test) along the manufacturing and storage. Control (C), 100% NaCl; F1, 50% NaCl; F2, 50% NaCl and 50% KCl; F3, 50% NaCl and 50% CaCl<sub>2</sub>; F4, 50% NaCl, 25% KCl, and 25% CaCl<sub>2</sub>.

directly related to the increased lipid oxidation observed in these treatments, since the formation of metmyoglobin due to oxidation during the process may have resulted in a darker color [37] for salamis with added CaCl<sub>2</sub>.

### 4. Conclusions

The results indicate that a reduction of 50% NaCl results in a reduction in lipid oxidation of dry fermented sausages during the curing process and shelf life. Furthermore, replacements of 50% NaCl with 50% CaCl<sub>2</sub> and with a blend of KCl and CaCl<sub>2</sub> were shown to have a negative effect on the oxidative stability of dry fermented sausages by increasing TBARS values and modifying fatty acid composition. In addition,

color parameters  $a^*$  and  $b^*$  were increased, while  $L^*$  and whiteness were decreased. Thus, from the point of view of the oxidative stability, we can conclude that the addition of KCl is a good alternative to reduce the NaCl content in fermented meat products.

#### **Competing Interests**

The authors declare that they have no competing interests.

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	D	Treatments					
	Days	Control	F1	F2	F3	F4	SEM <sup>a</sup>
	19/0	50.28 <sup>cd</sup>	51.49 <sup>bc</sup>	49.36 <sup>dB</sup>	53.36 <sup>aA</sup>	52.65 <sup>abA</sup>	0.30
$L^*$	30	50.84	51.43	51.18 <sup>A</sup>	52.08 <sup>B</sup>	$50.22^{B}$	0.25
L	60	50.34 <sup>b</sup>	51.24 <sup>a</sup>	49.51 <sup>bB</sup>	50.33 <sup>bC</sup>	$50.69^{abB}$	0.14
	90	50.28 <sup>cd</sup>	51.63 <sup>ab</sup>	50.79 <sup>bcAB</sup>	52.24 <sup>aC</sup>	$50.94^{bcB}$	0.17
	19/0	9.97 <sup>AB</sup>	10.38	$10.54^{AB}$	10.19 <sup>C</sup>	$10.41^{B}$	0.09
a*	30	10.35 <sup>bcA</sup>	10.80 <sup>abc</sup>	9.96 <sup>cB</sup>	$11.14^{abAB}$	11.37 <sup>aA</sup>	0.15
а	60	9.57 <sup>cB</sup>	10.27 <sup>b</sup>	9.96 <sup>bB</sup>	$10.84^{\mathrm{aB}}$	10.23 <sup>bB</sup>	0.08
	90	9.96 <sup>dAB</sup>	10.57 <sup>c</sup>	10.90 <sup>bcA</sup>	11.55 <sup>aA</sup>	$11.24^{abA}$	0.11
	19/0	6.14 <sup>bc</sup>	6.71 <sup>a</sup>	6.45 <sup>ab</sup>	6.88 <sup>aC</sup>	6.64 <sup>abC</sup>	0.08
b*	30	6.23 <sup>c</sup>	6.97 <sup>ab</sup>	6.67 <sup>bc</sup>	7.33 <sup>aB</sup>	7.17 <sup>aB</sup>	0.09
D	60	6.01 <sup>c</sup>	6.65 <sup>a</sup>	6.26 <sup>b</sup>	$7.04^{aAB}$	7.07 <sup>aB</sup>	0.07
	90	6.56 <sup>c</sup>	6.53 <sup>c</sup>	6.92 <sup>c</sup>	$7.90^{aA}$	7.85 <sup>abA</sup>	0.09
	19/0	48.92 <sup>cd</sup>	49.93 <sup>bc</sup>	47.87 <sup>dC</sup>	51.76 <sup>aA</sup>	51.06 <sup>abA</sup>	0.29
$\mathbf{W}$ hit are as $(0/)$	30	49.37	49.75	49.72 <sup>A</sup>	50.25 <sup>B</sup>	48.43 <sup>B</sup>	0.35
Whiteness (%)	60	49.07 <sup>ab</sup>	49.70 <sup>a</sup>	48.16 <sup>cBC</sup>	48.68 <sup>bcC</sup>	$49.15^{abB}$	0.14
	90	48.87 <sup>c</sup>	49.91 <sup>ab</sup>	49.12 <sup>bcAB</sup>	50.23 <sup>aB</sup>	49.06 <sup>bcB</sup>	0.16

TABLE 5: Color instrument ( $L^*$ ,  $a^*$ ,  $b^*$ , and whiteness) during storage of salamis with NaCl reduced or replaced by KCl and/or CaCl<sub>2</sub>.

<sup>a</sup>Standard error of the means (n = 9).

Different small letters in the same row indicate significant differences at P < 0.05 (Fisher's test). Different capital letters in each column for each parameter indicate significant differences at P < 0.05 (Fisher's test). Control, 100% NaCl; F1, 50% NaCl; F2, 50% NaCl and 50% KCl; F3, 50% NaCl and 50% CaCl<sub>2</sub>; F4, 50% NaCl, 25% KCl, and 25% CaCl<sub>2</sub>.

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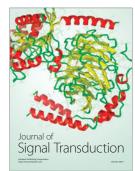






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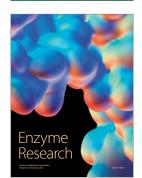


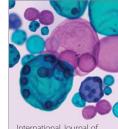
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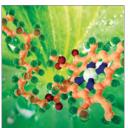
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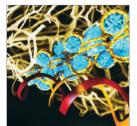


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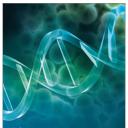
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