

## The impact of the partial replacement of sodium chloride in the development of starter cultures during Italian salami production

*Impacto da substituição parcial do cloreto de sódio no desenvolvimento da cultura starter durante a produção do salame tipo italiano*

Claudia Fieira<sup>1</sup>, João Francisco Marchi<sup>1</sup>, Daiana Marafão<sup>1</sup>, Alexandre da Trindade Alfaro<sup>1\*</sup>

<sup>1</sup>Universidade Tecnológica Federal do Paraná (UTFPR), Laboratório de Tecnologia de Alimentos, Francisco Beltrão/PR - Brasil

### \*Corresponding Author

Alexandre da Trindade Alfaro, Universidade Tecnológica Federal do Paraná (UTFPR), Laboratório de Tecnologia de Alimentos, Linha Santa Bárbara, s/n, Caixa Postal: 135, CEP: 85601-970, Francisco Beltrão/PR - Brazil, e-mail: alexandre@utfpr.edu.br

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### Abstract

Italian salami is a cured meat with high sodium contents which is easy, fast and convenient to consume. Starter cultures are used to improve its sensory characteristics and refine its technological manufacturing process. The goal of this study was to reduce the sodium content in Italian salami through the partial replacement of sodium chloride by potassium chloride, magnesium chloride and calcium chloride, and evaluate the viability of the *Lactobacillus* sp. and *Staphylococcus* sp. cells found in the starter culture. Four formulations were elaborated: one with, and one without the starter culture, but both with the addition of sodium chloride; and two with the partial replacement of 60% of the sodium chloride: the first with KCl, and the other with a mixture of KCl, MgCl<sub>2</sub> and CaCl<sub>2</sub>. Physicochemical and microbiological evaluations were carried out to monitor the ripening and the quality of the final product. The partial replacement of NaCl by other salts (MgCl<sub>2</sub>, CaCl<sub>2</sub>, KCl) did not interfere in the growth of the starter culture in the Italian salami, neither did it affect the majority of the physicochemical parameters of the Italian salami nor the microbiological quality of the final product.

**Keywords:** Cured sausages; Sodium content; *Staphylococcus*; *Lactobacillus*.

### Resumo

O salame tipo italiano, um embutido cárneo processado, de consumo fácil, prático e rápido, apresenta elevados teores de sódio. Para o seu processamento, utilizam-se culturas *starters* que melhoram suas características sensoriais e facilitam o processo tecnológico de fabricação. O objetivo deste trabalho foi reduzir o teor de sódio em salame tipo italiano, através da sua substituição parcial por cloreto de potássio, cloreto de magnésio e cloreto de cálcio, além de verificar a viabilidade das células de *Lactobacillus* sp. e *Staphylococcus* sp., presentes na cultura *starter*. Foram elaboradas quatro formulações: uma formulação sem e outra com cultura *starter*, ambas somente com adição de cloreto de sódio; outras duas, com substituição parcial de 60% do cloreto de sódio: uma por KCl e outra por mistura de KCl, MgCl<sub>2</sub>, CaCl<sub>2</sub>. Foram realizadas avaliações físico-químicas e microbiológicas para acompanhar o processo de maturação e a qualidade do produto final. A substituição parcial do NaCl por outros sais (MgCl<sub>2</sub>, CaCl<sub>2</sub>, KCl) não interferiu no desenvolvimento da cultura *starter* do salame tipo italiano. A maioria dos parâmetros físico-químicos do salame italiano não foi alterada, bem como a qualidade microbiológica do produto final.

**Palavras-chave:** Embutido cárneo; Teor de sódio; *Staphylococcus*; *Lactobacillus*.

### 1 Introduction

Excessive salt consumption represents a risk to certain groups of sensitive individuals prone to altered blood pressure, cardiovascular diseases, diabetes and kidney disease.

Arterial hypertension affects more than 25% of the world's adult population, representing greater risk for cardiovascular disease (TOLDRÁ; REIG, 2011). Sodium ingestion must be



## The impact of the partial replacement of sodium chloride in the development of starter cultures during Italian salami production

Fieira, C. et al.

restricted to less than 2,300 mg/day for non-hypertensive individuals and to between 1,500 mg and 2,300 mg/day for hypertensive individuals (MAGALHÃES et al., 2010).

The addition of sodium chloride (NaCl) to fermented meats is critical to guarantee their microbiological stability, flavour and texture, and it contributes to their basic distinctive flavour (TERRA et al., 2004). Generally, quantities of 2.0 to 4.0% of NaCl are added, and these values increase in the final product due to the drying process. In addition to NaCl, other salts, such as sodium nitrite (NaNO<sub>2</sub>), sodium nitrate (NaNO<sub>3</sub>) and sodium erythorbate (C<sub>6</sub>H<sub>7</sub>NaO<sub>6</sub>) are added, which are used to accelerate the cure, as well as to improve the texture, develop the distinctive flavour and eliminate the reheated flavour and antimicrobial activity, and these are also sources of sodium (ZANARDI et al., 2010).

Alternatives have been tested in cooked meat products, both fresh and smoked, including mixtures of different salts such as KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and potassium lactate (GIMENO et al., 2001; GELABERT et al., 2003; FULLADOSA et al., 2009; ARMENTEROS et al., 2009; VERMA et al., 2010; CARRARO et al., 2012; CIRIANO et al., 2013).

Starter cultures are used as a technological additive to manufacture fermented meat products, and are formed of numerous microbial species (IBAÑEZ et al., 1996). They are responsible for restricting the presence of undesirable microorganisms, reducing the manufacturing time, guaranteeing the homogeneity of the product, controlling the bacterial metabolism, improving the sensory characteristics and increasing the nutritional value, besides the ease of their technological applications (SHIMOKOMAKI et al., 2006). Several microbial species have been used in the preparation of fermented meat products with the purpose of providing the products with good sanitary quality (COELHO et al., 2009).

The salt concentration can affect the development of the starter culture and therefore the species selected for the preparation of fermented meat products must show good development under the processing conditions, including the temperature used and the presence of salt and nitrite (COELHO et al., 2009). Although the existing microorganisms reduce the nitrite concentration, the content of these compounds, together with the NaCl (both used in the curing salts), may affect the development of the starter cultures (HAULY et al., 2004).

Brazilian Normative no. 22 of July 31<sup>st</sup>, 2000, defines Italian salami as an industrialized meat product prepared with pork meat or a mixture of beef and pork meat, pork back fat, with the addition of additives and spices, ground to an average particle size between 6 mm and 9 mm, filled into a natural or artificial casing, cured and smoked or otherwise (BRASIL, 2000).

In this study, we attempted to reduce the sodium content of Italian salami by partially replacing the NaCl by KCl, MgCl<sub>2</sub> and CaCl<sub>2</sub>, as well as verifying the impact on the counts of the *Lactobacillus* sp. and *Staphylococcus* sp. cells found in the starter culture.

## 2 Material and methods

### 2.1 Material

Italian salami was prepared using the following ingredients: pork meat (boneless ham), beef (boneless sparerib) and pork back fat, salt (NaCl) (Diana, Curitiba, PR, Brazil), sucrose (crystal sugar), curing salt (93.75% NaCl and 6.25% NaNO<sub>2</sub> - Doremus Cura K001, Guarulhos, SP, Brazil), white pepper, garlic powder and nutmeg (local market), and sodium erythorbate (Doremus New Cor F014, Guarulhos, SP, Brazil).

The salts used were potassium chloride (KCl) (Vetec, Duque de Caxias, RJ, Brazil), magnesium chloride (MgCl<sub>2</sub>) (Nuclear, São Paulo, SP, Brazil) and calcium chloride (CaCl<sub>2</sub>) (Vetec, Duque de Caxias, RJ, Brazil). The reagents used in the analysis were PA analytical grade.

The starter culture used was TEXEL<sup>®</sup>AS-308 (Dupont Danisco, São Paulo, SP, Brazil) which contains the microorganisms *Lactobacillus sakei*, *Staphylococcus carnosus* and *Staphylococcus xylosus* with a total count of approximately 10.65 log CFU.g<sup>-1</sup>.

### 2.2 Preparation of the Italian salami

Four formulations of Italian salami were prepared (Table 1). The proportion of raw material, sodium chloride, sugars and spices used was based on the formulation for Italian salami described by Terra (2005) and Terra et al. (2004), with the replacement of 60% of the sodium chloride. The starter culture was not added to formulation F1; the NaCl was partially replaced (60%) by KCl in formulation F3 and by a mixture of KCl, MgCl<sub>2</sub> and CaCl<sub>2</sub> in formulation F4.

**Table 1.** Formulations used in the manufacture of Italian salami.

Ingredients	F1 (%)	F2 (%)	F3 (%)	F4 (%)
Pork meat	71	71	71	71
Beef	15	15	15	15
Pork back fat	10	10	10	10
Sucrose	0.3	0.3	0.3	0.3
Curing salt	0.3	0.3	0.3	0.3
White pepper	0.1	0.1	0.1	0.1
Garlic powder	0.1	0.1	0.1	0.1
Ground nutmeg	0.02	0.02	0.02	0.02
Sodium erythorbate	0.25	0.25	0.25	0.25
Starter culture	-	0.01	0.01	0.01
NaCl	2.5	2.5	1	1
KCl	-	-	1.5	0.5
CaCl <sub>2</sub>	-	-	-	0.5
MgCl <sub>2</sub>	-	-	-	0.5

## The impact of the partial replacement of sodium chloride in the development of starter cultures during Italian salami production

Fieira, C. et al.

The pork meat, beef and pork back fat were ground using an 8 mm disc. The ingredients were mixed manually, adding the salts first in order to extract the myofibrillar proteins. The starter culture was mixed with 50 ml of distilled water, left to stand for 30 minutes and then added to the formulation. The others ingredients were added in sequence.

After mixing the mass was filled into collagen casings (40 mm).

The Italian salamis were stored in an industrial refrigerator at 7 °C for 12 hours, followed by smoking for 6 hours in an oven (Arprotec, Valinhos, SP, Brazil) at temperatures between 28 °C and 35 °C, with a relative humidity of 85% and the injection of natural smoke. After curing, the salamis were incubated in a B.O.D. chamber (Logen, LS334, São Paulo, SP, Brazil), for 28 days (ripening period). The range of relative humidity was 75% to 95%, and of temperature 18 °C to 25 °C according to the stage of ripening.

### 2.3 Physicochemical analyses

The physicochemical analyses of the formulations (F1, F2, F3 and F4) were carried out on days 0, 3, 7, 14, 21 and 28. All the evaluations were carried out in triplicate.

The pH value was determined to monitor the fermentation stage (TERRA et al., 2004) using a MS Tecpon instrument pH meter (mPA-210, Cachoeirinha, RS, Brazil). Gravimetric methods were used to evaluate the moisture content (IAL, 2008), and weight loss, by weighing a piece of sausage (MACEDO et al., 2008).

The moisture (gravimetric method), protein (Micro-Kjeldahl method), fat (Soxhlet) and ash (mineral residue by incineration) contents (IAL, 2008) were evaluated at the end of the maturation period of the salamis. The percent of carbohydrates was calculated as the difference between 100 and the sum of the protein, lipid, moisture, ash and chloride contents (BRASIL, 1998).

The water activity ( $A_w$ ) of the samples was determined with the use of a water activity measurer at 25 °C (Novasina, Lab Master, Lachen, Switzerland) and the percentage of chlorides was determined by the Mohr method (LANARA, 1981).

The instrumental colour was determined on the 28<sup>th</sup> day of ripening, on the surface of the inner part of the samples. A colorimeter (KONICA MINOLTA CR 400/410, Osaka, Japan) was used to determine the CIELab coordinates:  $L^*$  (luminosity or percent reflectance, ranging from black 0% to white 100%),  $a^*$  (variation between the colours green  $-a^*$  and red  $+a^*$ ), and  $b^*$  (variation between blue  $-b^*$  and yellow  $+b^*$ ), using illuminant D65 and an observation angle of 10° (NASCIMENTO et al., 2007).

### 2.4 Microbiological analyses

The microbiological analyses were carried out on days 0, 3, 7, 14, 21 and 28. All evaluations were carried out in triplicate.

The *Lactobacillus* sp. count was done on MRS agar (Merck Chemicals, Darmstadt, Germany) and the *Staphylococcus* sp. count on Baird Parker agar (Himedia, Mumbai, India) with incubation at 37 °C for 48 hours. The results were expressed in CFU g<sup>-1</sup> (MACEDO et al., 2008).

At the end of the maturation period, microbiological quality of the salamis was evaluated according to the requirements of Instruction RDC no. 12 (BRASIL, 2001), which requires the determinations of the fecal coliform and coagulase-positive *Staphylococcus* counts and the determination of the presence or absence of *Salmonella* sp, all carried out according to the procedures described by Brasil (2003).

### 2.5 Statistical analysis

The data obtained in the analyses of the Italian salamis were subjected to the analysis of variance (ANOVA), and the means compared by Tukey's test, using a significance level of 5% ( $p < 0.05$ ) and the Statistica 7.0 software (StatSoft Inc., Tulsa, Oklahoma).

## 3 Results and discussion

### 3.1 Evaluation of the salamis during the ripening stage

#### 3.1.1 *Lactobacillus* sp. count

The *Lactobacillus* sp. count (Table 2) was carried out to monitor the viable cells throughout the maturation process, verifying the impact of the replacement of 60% of the NaCl on the development of the starter culture.

Formulations F2 and F4 showed the highest number of viable *Lactobacillus* sp. cells on the day of manufacture (time 0) ( $p \leq 0.05$ ). Aliño et al. (2009) did not find significant differences in the counts of aerobic mesophilic and lactic

**Table 2.** *Lactobacillus* sp. count (log CFU.g<sup>-1</sup>) in Italian salamis produced with different sodium contents during their ripening.

Time (day)	F1	F2	F3	F4
0	3.04 ± 0.05 <sup>c</sup>	6.01 ± 0.00 <sup>a</sup>	5.89 ± 0.01 <sup>b</sup>	6.02 ± 0.02 <sup>a</sup>
3	3.14 ± 0.10 <sup>c</sup>	4.40 ± 0.65 <sup>b</sup>	6.00 ± 0.00 <sup>a</sup>	6.00 ± 0.00 <sup>a</sup>
7	3.56 ± 0.17 <sup>c</sup>	5.64 ± 0.04 <sup>b</sup>	5.54 ± 0.06 <sup>b</sup>	6.00 ± 0.00 <sup>a</sup>
14	5.99 ± 0.01 <sup>a</sup>	5.98 ± 0.00 <sup>a</sup>	5.98 ± 0.02 <sup>a</sup>	6.00 ± 0.00 <sup>a</sup>
21	3.99 ± 0.00 <sup>c</sup>	5.82 ± 0.03 <sup>b</sup>	5.99 ± 0.01 <sup>a</sup>	6.00 ± 0.00 <sup>a</sup>
28	3.23 ± 0.06 <sup>d</sup>	4.17 ± 0.04 <sup>c</sup>	5.13 ± 0.01 <sup>a</sup>	4.73 ± 0.19 <sup>b</sup>

Means ± standard deviation; means followed by the same letter in the same line, do not represent a significant difference ( $p \leq 0.05$ ). F1: NaCl; F2: NaCl + starter culture; F3: NaCl + KCl + starter culture; F4: NaCl + KCl + CaCl<sub>2</sub> + MgCl<sub>2</sub> + starter culture.

## The impact of the partial replacement of sodium chloride in the development of starter cultures during Italian salami production

Fieira, C. et al.

acid bacteria amongst dry cured loin formulations with different salt concentrations. However, Blesa et al. (2008) observed that the type of salt mixture used in the salting process might affect the viability of these bacteria in cured ham.

Formulation F4 (replacement of 60% of the NaCl by KCl, MgCl<sub>2</sub> and CaCl<sub>2</sub>) exhibited higher viable cell counts throughout the ripening period, although the number was lower than that in Formulation F3 (replacement of 60% of the NaCl by KCl) at the end of the process. Formulations F1 and F2 showed the lowest *Lactobacillus* sp. cell counts on the 28<sup>th</sup> day. The partial replacement of sodium chloride (F3 and F4) by KCl, MgCl<sub>2</sub> and CaCl<sub>2</sub> did not affect the growth of *Lactobacillus* sp.

The *Lactobacillus* sp. counts in the Italian salamis corroborated the results obtained by Macedo et al. (2008), who verified that all the strains of *Lactobacillus* (*L. casei*, *L. paracasei* ssp. *paracasei* and *L. casei* ssp. *Rhamnosus*) tested showed a high number of viable cells when cultured in MRS agar.

The results showed that the partial replacement of sodium chloride did not interfere with the activity of the *Lactobacillus* sp. This is desirable because the physiological activity of the microorganisms causes desirable transformations during the meat fermentation process, which influence the characteristics of the final product (SAWITZKI, 2007).

### 3.1.2 *Staphylococcus* sp. count

On the day of manufacture of the Italian salamis (time 0), the *Staphylococcus* sp. counts differed amongst the salami formulations ( $p \leq 0.05$ ) (Table 3). Formulation F2 showed the highest count when compared to the other formulations, while formulation F4 exhibited the lowest *Staphylococcus* sp. count.

It is important to highlight that the formulations in which 60% of the NaCl was replaced by other salts, presented significantly ( $p \leq 0.05$ ) lower *Staphylococcus* sp counts. The higher concentration of sodium chloride

in formulation F2 might have improved the growth of *Staphylococcus* sp. These results are in agreement with Gonçalves (2013), who observed the tolerance of *Staphylococcus carnosus* to NaCl concentrations above 15% and the fact that *Staphylococcus xyloso* showed optimal growth in a culture medium containing 10% NaCl

Studies by Mauriello et al. (2004) and Casaburi et al. (2005) demonstrated that *Staphylococcus carnosus* and *Staphylococcus simulans* grew at temperatures of 15 °C and 20 °C (temperatures usually used for meat fermentation) in the presence of 10%, 15% or 20% of NaCl, at pH values of 5.0 and 5.5. Gøtterup et al. (2007) also reported that *S. xyloso* showed optimum growth at 30 °C, pH 5.5 with 20% of NaCl.

At the end of the ripening process, formulation F3 showed the highest viable cell counts, exceeding the counts in formulation F2. This could be related to the lower pH values found in this formulation at the end of the maturation process. According to Gonçalves (2013), low pH values are adverse to the growth of *Staphylococcus*.

The presence of a high *Lactobacillus* sp. count in formulation F4 (Table 2) during maturation decreased the pH of the salami. This lower pH value probably caused the reduction in the number of *Staphylococcus* sp. since pH values below 5.7 adversely affect the development of this microorganism (STAHNKE, 1995). Papamanoli et al. (2003) and Macedo et al. (2008) working with fermented meats, also observed a drop in pH, and related this to the high number of probiotic *Lactobacillus* cells.

The *Staphylococcus* sp. counts in formulation F3, with partial replacement of the NaCl by KCl, were higher, demonstrating that the presence of this salt did not influence the development of the microorganism.

### 3.1.3 pH

During the first 14 days of maturation the pH of the formulations varied from 4.99 to 5.51, and after this, from 4.98 to 5.8. According to Terra et al. (2004), lower pH values help the homo-fermentative lactic bacteria to exceed the contaminants due to competitive antagonism, besides providing the necessary conditions for the reduction of nitrate to nitrite in order to create nitrous myoglobin. In addition the drop in pH must occur gradually up to the seventh day, reaching values around 5.0, decreasing from the initial values of 5.8-6.0 to 5.0-5.2 in raw meats.

Formulation F1, with no starter culture, showed an initial pH of 5.54. This slowly decreased and then started to increase again after the 21<sup>st</sup> day, finally reaching a pH of 5.64. Formulation F2, with starter culture, showed an initial pH of 5.81 which rapidly decreasing to 4.84, subsequently increasing to 4.93 and finally reaching 5.65. The use of the starter culture allowed for faster acidification, avoiding the development of undesirable microorganisms, improving

**Table 3.** *Staphylococcus* sp. count (log CFU.g<sup>-1</sup>) in Italian salamis produced with different sodium contents during ripening.

Time (day)	F1	F2	F3	F4
0	5.66 ± 0.05 <sup>ab</sup>	5.99 ± 0.01 <sup>a</sup>	4.60 ± 0.25 <sup>c</sup>	5.33 ± 0.05 <sup>b</sup>
3	4.38 ± 0.35 <sup>b</sup>	5.10 ± 0.11 <sup>a</sup>	5.15 ± 0.12 <sup>a</sup>	4.67 ± 0.15 <sup>ab</sup>
7	6.00 ± 0.00 <sup>a</sup>	6.00 ± 0.00 <sup>a</sup>	5.98 ± 0.02 <sup>a</sup>	5.81 ± 0.05 <sup>b</sup>
14	5.93 ± 0.09 <sup>a</sup>	5.60 ± 0.16 <sup>b</sup>	6.00 ± 0.02 <sup>a</sup>	5.83 ± 0.04 <sup>ab</sup>
21	4.52 ± 0.06 <sup>b</sup>	5.99 ± 0.03 <sup>a</sup>	6.00 ± 0.00 <sup>a</sup>	5.95 ± 0.02 <sup>a</sup>
28	5.81 ± 0.02 <sup>a</sup>	5.33 ± 0.03 <sup>b</sup>	6.00 ± 0.00 <sup>a</sup>	4.54 ± 0.23 <sup>c</sup>

Means ± standard deviation; averages followed by the same letter in the same line do not present a significant difference ( $p \leq 0.05$ ). F1: NaCl; F2: NaCl + starter culture; F3: NaCl + KCl + starter culture; F4: NaCl + KCl + CaCl<sub>2</sub> + MgCl<sub>2</sub> + starter culture.

## The impact of the partial replacement of sodium chloride in the development of starter cultures during Italian salami production

Feira, C. et al.

the colouration, speeding up dehydration and adding an acid flavour to the product (TERRA, 2005).

Formulations F3 and F4, with partial replacement of 60% of the sodium chloride, showed lower initial pH values in comparison with formulation F2 but also showed rapid acidification of the medium although sample F4 showed a lower final pH. Stahnke (1995) found a similar result in sausages with low sodium chloride contents (1% to 5%), where the pH was reduced at high fermentation temperatures (15 °C to 25 °C) and lower salt contents of.

The pH values found during the maturation period corroborated the results obtained by Campagnol et al. (2011), which can be considered normal for this type of meat product. Guardiola et al. (2008) replaced 50% of the NaCl by KCl in salamis and found no significant differences between the treatments.

### 3.1.4 Weight loss and moisture

The weight loss from salamis indicates the loss of water and also of important water-soluble substances during fermentation. Acidification occurs at this stage, and most of the water is released with proximity to the isoelectric point of the myofibrillar proteins. Dehydration is essential to the safety and quality of the product, also affecting other sensory characteristics (TERRA, 2005). Table 4 shows the average values obtained for weight loss and moisture content.

There was considerable weight loss during the maturation process of the Italian salamis, with a significant difference between treatments ( $p \leq 0.05$ ). The difference in weight loss between the F2 treatment, with starter culture, and the F1 treatment, without starter culture, was 3%.

The replacement of 60% of the NaCl in treatments F3 and F4 caused increases of 7% and 5%, respectively, in weight loss at the end of the process in relation to the F2 treatment. According to Sgarbiere (1998), this difference occurs because the addition of salt to meat products increases the ionic strength, improving the solubility and consequently the functionality of the myofibrillar proteins. Depending on the salt concentration of the medium, the meat proteins can either retain or release water.

There was a considerable variation in moisture content between treatments during the maturation process ( $p \leq 0.05$ ), treatments F4 and F1 showing the highest moisture losses. The replacement of 60% of the NaCl by KCl caused a 2% reduction in the moisture content and 6% reduction when replaced by the salt mixture at the end of the process. These results are in agreement with Guardiola et al. (2008), who observed a significant difference in the moisture content of salami when 50% of the NaCl was replaced by KCl, and with Aliño et al. (2009), when they replaced 70% of the NaCl by KCl in cured and dry loin.

## 3.2 Characterization of the final product

### 3.2.1 Proximal composition, Aw and pH

The standards of identity and quality for Italian salamis determine the following product parameters: water activity of 0.90; maximum moisture content of 35%; maximum fat content of 32%; minimum protein content of 25%; total carbohydrate content of 4% (BRASIL, 2000).

Table 5 shows the values obtained for the proximate composition, water activity and final pH of the salamis.

With regards to moisture content, only formulation F2 showed a value above that determined by the legislation (37%).

**Table 4.** Weight loss and moisture content of Italian salamis produced with different sodium contents, during maturation (days).

Time (day)	Weight Loss (%)				Moisture content (%)			
	F1	F2	F3	F4	F1	F2	F3	F4
3	13 ± 0.33 <sup>a</sup>	10 ± 0.47 <sup>b</sup>	13 ± 0.70 <sup>a</sup>	13 ± 0.49 <sup>a</sup>	NA	NA	NA	NA
7	13 ± 0.33 <sup>b</sup>	11 ± 0.81 <sup>c</sup>	16 ± 1.40 <sup>a</sup>	16 ± 0.49 <sup>a</sup>	85 ± 0.57 <sup>a</sup>	87 ± 0.57 <sup>a</sup>	75 ± 0.57 <sup>c</sup>	82 ± 0.57 <sup>b</sup>
14	27 ± 0.33 <sup>c</sup>	25 ± 0.81 <sup>d</sup>	29 ± 0.70 <sup>b</sup>	32 ± 0.49 <sup>a</sup>	72 ± 0.40 <sup>b</sup>	75 ± 0.40 <sup>a</sup>	61 ± 0.40 <sup>d</sup>	68 ± 0.40 <sup>c</sup>
21	37 ± 0.33 <sup>b</sup>	34 ± 1.24 <sup>b</sup>	41 ± 0.70 <sup>a</sup>	42 ± 1.24 <sup>a</sup>	52 ± 0.44 <sup>b</sup>	57 ± 0.44 <sup>a</sup>	43 ± 0.44 <sup>c</sup>	51 ± 0.44 <sup>b</sup>
28	44 ± 0.33 <sup>c</sup>	41 ± 0.94 <sup>d</sup>	48 ± 0.70 <sup>a</sup>	46 ± 0.47 <sup>b</sup>	34 ± 1.28 <sup>ab</sup>	37 ± 1.28 <sup>a</sup>	35 ± 1.28 <sup>ab</sup>	31 ± 1.28 <sup>b</sup>

Means ± standard deviation; three repetitions; averages followed by the same letter in the same line do not show a significant difference ( $p \leq 0.05$ ). F1: NaCl; F2: NaCl + starter culture; F3: NaCl + KCl + starter culture; F4: NaCl + KCl + CaCl<sub>2</sub> + MgCl<sub>2</sub> + starter culture; NA=not evaluated.

**Table 5.** Moisture, ash, lipid, protein, chloride and carbohydrate contents (%), and the Aw and pH values of Italian salamis produced with different sodium contents, after 28 days of ripening.

Formulation	Moisture	Ash	Lipid	Protein	Chloride	Carbohydrate	Aw	pH
F1	35.0 ± 0.8 <sup>ab</sup>	7.0 ± 0.0 <sup>a</sup>	13.5 ± 0.1 <sup>c</sup>	30.6 ± 0.3 <sup>a</sup>	5.3 ± 0.1 <sup>a</sup>	8.3 ± 0.6 <sup>ab</sup>	0.89 ± 0.0 <sup>a</sup>	5.6 ± 0.0 <sup>a</sup>
F2	37.7 ± 0.6 <sup>a</sup>	7.0 ± 0.2 <sup>a</sup>	14.5 ± 0.1 <sup>b</sup>	32.2 ± 0.9 <sup>a</sup>	5.0 ± 0.0 <sup>a</sup>	3.4 ± 1.1 <sup>bc</sup>	0.89 ± 0.0 <sup>a</sup>	5.6 ± 0.0 <sup>a</sup>
F3	35.3 ± 1.1 <sup>ab</sup>	7.5 ± 0.5 <sup>a</sup>	19.8 ± 0.0 <sup>a</sup>	31.3 ± 0.8 <sup>a</sup>	5.2 ± 0.0 <sup>a</sup>	0.6 ± 1.2 <sup>c</sup>	0.89 ± 0.0 <sup>a</sup>	5.8 ± 0.0 <sup>a</sup>
F4	31.2 ± 1.9 <sup>b</sup>	6.3 ± 0.0 <sup>a</sup>	11.6 ± 0.3 <sup>d</sup>	33.0 ± 0.5 <sup>a</sup>	5.5 ± 0.0 <sup>a</sup>	12.1 ± 2.8 <sup>a</sup>	0.89 ± 0.0 <sup>a</sup>	4.9 ± 0.0 <sup>b</sup>

Mean ± standard deviation; three replicates; averages followed by the same letter in the same column do not show a significant difference ( $p \leq 0.05$ ). F1: NaCl; F2: NaCl + starter culture; F3: NaCl + KCl + starter culture; F4: NaCl + KCl + CaCl<sub>2</sub> + MgCl<sub>2</sub> + starter culture.

## The impact of the partial replacement of sodium chloride in the development of starter cultures during Italian salami production

Fieira, C. et al.

**Table 6.** Mean values for L\*, a\* and b\* in Italian salamis produced with different sodium contents, after 28 days of maturation.

Colour coordinate	F1	F2	F3	F4
L*	38.00 ± 1.29 <sup>b</sup>	44.03 ± 1.60 <sup>ab</sup>	44.32 ± 2.85 <sup>ab</sup>	48.96 ± 0.71 <sup>a</sup>
a*	15.06 ± 0.51 <sup>a</sup>	14.18 ± 0.76 <sup>a</sup>	14.34 ± 1.75 <sup>a</sup>	12.90 ± 1.73 <sup>a</sup>
b*	7.94 ± 0.21 <sup>a</sup>	8.82 ± 0.42 <sup>a</sup>	9.53 ± 0.66 <sup>a</sup>	9.77 ± 1.20 <sup>a</sup>

Means ± standard deviation; three repetitions; means followed by the same letter in the same line do not show a significant difference ( $p \leq 0.05$ ). F1: NaCl; F2: NaCl + starter culture; F3: NaCl + KCl + starter culture; F4: NaCl + KCl + CaCl<sub>2</sub> + MgCl<sub>2</sub> + starter culture.

The quantities of ash and protein showed no significant differences ( $p \leq 0.05$ ) between treatments. With respect to the lipid contents, all the formulations showed significant differences ( $p \leq 0.05$ ), but the protein contents of the four formulations were within the amounts allowed.

The Aw values obtained for the four formulations were close to the amounts required by the legislation. For the pH measurements, formulation F4 showed significantly different values ( $p \leq 0.05$ ) than the other formulations.

Formulation F3, with partial replacement of the NaCl by KCl showed lower values for the moisture, protein and carbohydrate contents and for Aw, and higher values for the ash and lipid contents and for pH, in comparison with formulation F2. Formulation F4, in which the NaCl was partially replaced by a salt mixture, showed lower values for the moisture, ash and lipid contents and for pH, and higher values for the protein and carbohydrate contents, in comparison to formulation F2.

Rech (2010) evaluated different formulations of Italian salami with reduced sodium contents and partial replacement with other salts. The author stated that variations in the chemical composition of the product occurred due to specific variables of the maturation process, such as the position of the stick and the position in the maturation chamber, amongst others.

Most of the physicochemical parameters of the Italian salami produced with reduced sodium content were not altered. Similar results were obtained by Vogel et al. (2011) in sausages, Gelabert et al. (2003) in fermented meats, Aliño et al. (2009) in cured dry lard, and Barbosa (2009) in Hamburg salami.

### 3.2.2 Instrumental colour

The treatments did not show significant differences ( $p \leq 0.05$ ) for the colour coordinates a\* and b\* at the end of ripening (Table 6). A similar result was found by Aliño et al. (2009), who found no significant differences ( $p > 0.05$ ) in the a\* and b\* values of cured and dry loin with the replacement of 35%, 50% and 70% of NaCl by KCl.

The luminosity values were higher in formulation F4. According to Aliño et al. (2010), higher calcium and magnesium contents, and differences in the water content, pH and additives can cause alterations in the luminosity (L\*).

### 3.2.3 Microbiological characterization of the final product

In order to evaluate the microbiological quality of the treatments after 28 days, the following analyses were performed: fecal coliforms, positive coagulase Staphylococcus, and Salmonella sp. According to the Brazilian legislation (Brasil, 2001), the maximum limit for the count of fecal coliforms, positive coagulase Staphylococcus and Salmonella sp. in fermented meats are 10<sup>3</sup> CFU/g, 5x10<sup>3</sup> in CFU/g and absence in 25g of the product. Regarding these standards, salami of all treatments met the Legislation requirements.

## 4 Conclusion

The partial replacement of NaCl by other salts (MgCl<sub>2</sub>, CaCl<sub>2</sub>, KCl) did not interfere in the growth of the starter culture in Italian salami. The growth of *Lactobacillus* sp. was improved by the partial replacement of NaCl by MgCl<sub>2</sub>, CaCl<sub>2</sub> or KCl. The partial replacement of NaCl did not affect most of the physicochemical parameters of the Italian salami or the microbiological quality of the final product.

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**The impact of the partial replacement of sodium chloride in the development of starter cultures during Italian salami production**

Fieira, C. et al.

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**The impact of the partial replacement of sodium chloride in the development of starter cultures during Italian salami production**

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