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Free-sodium salts mixture and AlgySalt® use as NaCl substitutes in fresh and cooked meat products intended for the hypertensive population

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ABSTRACT

This work aims at reducing the use of added NaCl in processed meat products because of its negative effects on hypertensive population by replacing it by sodium-free salts mixture (SM: KCl, $MgCl_2$ and $CaCl_2$) in fresh and cooked sausages. The technological, sensory, and microbiological effects of SM were compared with a commercial replacer based on seaweed extracts (AlgySalt®). A total substitution of NaCl with the latter and a partial one with SM (80% and 50%) were studied in cooked sausages and a total NaCl substitution with both substitutes was performed in fresh sausages. As a result, hardness increased in AlgySalt® reformulated samples, while it decreased when 80% SM were used. Whereas, AlgySalt® induced less cooking loses than SM. To some extent, microbiological counts showed a similarity between reformulated and control samples for both sausage types, whereas reformulated products containing SM revealed better sensory properties for both meat products. Therefore, using SM as NaCl replacer is adequate for processed meat products.

1. Introduction

In the recent decades, the agro-food sector has registered a much accelerated growth mainly thanks to the technological innovation of its products, which was mainly based on their organoleptic acceptability by the consumers. This growth is primarily due to the evolution of lifestyles, urbanization, and habits which are heading more towards the consumption of processed foods. However, in order to be well accepted by the consumers, the industry promotes food products containing high amounts of *trans* acids, saturated fat, sugars, and salt (NaCl). The latter is the main source of sodium, which, when overconsumed, causes an increase in blood pressure and leads to several complications either at heart muscle or arteries level (Doyle & Glass, 2010). Moreover, a large percentage of the World's population has a genetic predisposition to high blood pressure which is being caused by weight excess and high sodium consumption. Several scientific studies such as those by Doyle and Glass (2010) and Haizhou et al. (2015) showed that over-consumption of sodium and insufficient absorption of potassium (< 3.5 g/day, (WHO, 2016)) contribute to the appearance of non-communicable diseases NCD such as high blood pressure, cardiovascular disease (CVD) and strokes. These are the main causes of morbidity (45% of CVD are caused by hypertension) and mortality (13% of deaths) in the world. Salt can become harmful when consumed in amounts which exceed the recommended daily intake of 5 g/day, equivalent to 2 g/day of sodium (WHO, 2016). It should be noted that one gram of sodium chloride (NaCl) contains 0.40 g of Na (i.e. 17 mEq). However, the daily food intake in Western countries provides 9 to 12 g of NaCl, 3.6 to 4.8 g of Na (172–258 mEq) which is twice the recommended maximum level of intake.

Therefore, a reduction in NaCl consumption has been recently recommended by qualified international institutions such as the NAOS (2009) strategies. The latter published a report analyzing the main sources of sodium in the diet, among which meat products are the most represented ones. Indeed, Meat derivatives have high concentrations of sodium, including transformed meat products such as cooked and fresh ones (about 1720 mg/100 g and 925 mg/100 g, respectively) (ANSES, 2013). The estimates indicate that approximately 30% of the dietary sodium comes from the consumption of meat and its products (NAOS, 2009), although sodium is relatively low in meat (contains < 100 mg of Na per 100 g [Ruusunen & Puolanne, 2005]). Several publications put emphasis on the importance of sodium content reduction and its impact on techno functional and the food organoleptic properties of different

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types of meat products (Gelabert, Gou, Guerrero, & Arnau, 2003). As to our knowledge, there are few studies on the reduction of sodium in fresh meat products (Pasin et al., 1989; Triki, Herrero, Jiménez-Colmenero, & Ruiz-Capillas, 2013). However, significant efforts have been made to reduce the contents of Na in fermented, matured, and cooked meat products (Aaslyng, Vestergaard, & Koch, 2014; Ruusunen et al., 2005). These strategies are essentially based on either simple reduction or partial substitution of sodium chloride with other compounds that might produce similar effects to NaCl. Depending on the product type, these substitutions revealed different problems mainly because salt (NaCl) is essential for the preparation of meat products as it has various functions on the texture, flavor, and microbial development (Weiss, Gibis, Schuh, & Salminen, 2010). To address these primary functions of salt, different alternatives have been studied a case in point is the use of KCl. Nevertheless, the concentration of the latter was limited to 0.5–0.6% because of its bitter and metallic flavors that can reduce sensory properties (Desmond, 2006). Other compounds were incorporated such as phosphate and organic acid salts that are associated with other ingredients and additives, such as proteins, dietary fibers, hydrocolloids, and starches. The latter was used as supplements to alternative salts to improve the stability of the products, because they have a high water and fat retention capacity (Ruiz-Capillas & Jiménez-Colmenero, 2009; Ruusunen & Puolanne, 2005; Totosaus & Perez-Chabela, 2009; Verma, Sharma, & Banerjee, 2010). According to the existing literature, the decrease in the level of sodium in meat products through NaCl substitution by a mixture of salts of potassium, calcium, and magnesium yielded very satisfying results (Armenteros, Aristoy, & Toldrá, 2009; Gimeno, Astiasarán, & Bello, 2001; Triki et al., 2013; Zanardi, Ghidini, Conter, & Ianieri, 2010). Therefore, it would be interesting to study this type of reduction in processed meat products offered to consumers.

In this context, the objective of this study is to reduce sodium level in meat products through the substitution of added NaCl by a mixture of KCl, $CaCl_2$ and $MgCl_2$ salts (SM). Moreover, the effects of SM use will be compared to the one of a commercial substitute which is an extracted from seaweed (AlgySalt®). The studied meat derivatives are processed fresh and cooked products that will be compared to the reformulated products in terms of sensory, technological and microbiological attributes. AlgySalt® was chosen as one of the studied substitutes because of its natural and innovative composition in seaweed extracts, which is totally different from SM, as well as its commercial appeal to the international market (a wide range of countries from the European, Asian and African continents).

2. Material and methods

2.1. Fresh and cooked meat sausages manufacturing

2.1.1. Fresh meat sausages

Fresh meat sausages were made from minced turkey meat and beef fat as raw material. Other ingredients were added such as 3.9% of spices (including 0.5% of coriander [Naturel, Conditionnement de produits agricoles, El Sahlin, Tunisia], 0.8% of fennel, 0.2% of paprika, 0.2% of hot pepper, and 0.2% of mint [the four spices were obtained from Kamy S.A., Nabeul Tunisia] and 2.0% of a commercial preparation of Harissa [Le phare du Cap bon Ferrero, SCAPCB S.A., Grombalia, Tunisia]. The harissa paste is composed of 87% of chili pepper, 4% of garlic, 4% of coriander, 3% of salt, and 2% of caraway), water, NaCl, and 2.254% of additives (including 0.004% of E250 sodium nitrite, 0.03% of E120 dye, and 2.22% of E1422 modified starch all of them purchased from Sigma Aldrich, France). AlgySalt® was used for the reformulated products. It is a seaweed mixture including an ensemble of algae which are *Lithothamnium calcareum*, *Laminaria*, *Enteromorpha*, *Ascophyllum nodosum*, *Palmaria palmata*, *Fucus vesiculosus*, *Himanthalia elongata*, *Laminaria saccharina*, *Ulva lactuca*, *Chondrus crispus*, *Porphyra umbilicalis*, Palmaria/Porphyra/Ulva, and *Undaria pinnatifida*. This product also contains minerals, hydrocolloids, and fibers of the aforementioned seaweeds origin. First, minced meat (70%) and fat (20%) were mixed in a cutter (GARANT 35 H Bowl Chopper, floor-mounted, Germany). Then the ingredients and additives (6.154%) were added uniformly, including NaCl (0.846%) (for control samples), during 5 min at 4 °C. Finally, water (3%) was added to the final mixture. Four formulations were manufactured: a control sample with 0.846% of NaCl (FC); a total NaCl substitution with a Salt Mixture (SM: 50% KCl, 35% $MgCl_2$, and 15% $CaCl_2$) (FSM); a total NaCl substitution with AlgySalt® (FA); and a sample without NaCl (FN), which was substituted by water. The later was chosen as a NaCl replacer for different reasons. The main ones were the following: a) The FN formulation was used as a positive control in terms of NaCl total absence; and b) In order to have results that can be interpreted, the experimental design took into consideration that water is a non-meat ingredient which is present in all formulations as well as being the major component (moisture) of the fresh sausages (Triki et al., 2013). Therefore no additional variables would interfere in the discussion of our findings. The embossing was conducted manually into a 22 mm-diameter natural casings (mutton origin). The fresh meat products were stored at 4 °C in EPS trays (Type 89 white SPT—Linpac Packaging Pravia, S.A. N R.G.S., Spain) and covered with oxygen-permeable cling film (LINPAC Plastics, Pontivy, France) in aerobic conditions during 7 days. This manufacturing process was repeated three times to assure the process repeatability. Thus, three separate independent batches of the base sausage mixture were prepared. Every manufactured formulation yielded an average of 190 sausages per formulation.

2.1.2. Cooked meat sausages

Cooked meat sausages were made from mechanically separated turkey meat (MSM) as raw material. Other additives (including 0.002% of E450 diphosphate, 0.002% of E451 triphosphate, 0.002% of E452 polyphosphate, 0.04% of E120 dye, 8.15% of E1422, 0.094% of E300, and 0.01% of E250 sodium nitrite purchased from Sigma Aldrich, France) and ingredients (including 0.85% of paprika and 0.85% of hot pepper obtained from Kamy S.A., Nabeul Tunisia) were used for the formulation as well as AlgySalt® as one of the substitutes of NaCl.

The Protocol for the manufacturing of the cooked meat product was similar to the one used for fresh meat sausage with the exception using mechanically separated meat (MSM) (80%) instead of minced meat. Then, additives and ingredients (10%) as well as NaCl (2%) (for control samples) and water (8%) were added and mixed using a cutter (GARANT 35 H Bowl Chopper, floor-mounted, Germany). The embossing was made in 58 mm-diameter opaque Polyvinylidene chloride (PVDC) artificial casings (Krehalon, France). After that, the embossed sausages underwent a cooking stage at 80 °C for 30 min in a water bath (Haake L, Haake Buchler Instruments, Karlsruhe, Germany). A thermocouple was set in the center of the sample to follow the product core temperature. Afterwards, sausages were first cooled in a mixture of cold water and ice until core temperature reached < 10 °C and then stored at 4 °C during 15 days. Four formulations were developed: a control sample with 2% of NaCl (CC); two partial NaCl replacements with the SM, one sample with 50% substitution of the NaCl (C50) and another one with 80% substitution (C80); and a total NaCl substitution with AlgySalt® (CA). The manufacturing process was repeated three times to assure the process repeatability. Thus, three separate independent batches of the base sausage mixture were prepared. Every manufactured formulation yielded an average of 95 sausages per formulation.

2.2. Physicochemical properties

2.2.1. Mineral composition

For minerals determination (Na, Ca, and K), we used the methodology of Serrano et al. (2005). Mineral content was expressed in mg/kg of the product that is obtained through an atomic absorption

spectrophotometer (AAS Zeenit 700 Analytikjena). Briefly, after placing 1 g of sample in a previously weighed crucible (P0) and measuring the weight (P1), the crucible was placed in an oven at 105 °C for 24 h. Afterwards, the weight was measured again (P2). The sample was then put in an oven at 550 °C for 24 h to get the ashes of the product which were in turn weighted (P3). The ashes were treated with nitric acid for digestion in order to minimize the losses of some minerals by evaporation. The last step was to prepare the different dilutions for spectrometry atomic absorption.

2.2.2. pH

The pH was determined by using a pH meter (METROHM 744 pH meter) for solid samples at ambient temperature. Prior to use, the pH meter was calibrated using three Metrohm buffer solutions whose pHs were 4, 7, and 9, at room temperature.

2.2.3. Cooking loss (water and fat binding properties)

The analysis of cooking loss was performed following the methodology of Triki et al. (2013) to study the binding properties of water and fat. Briefly, 15 g of each formulation were put in Falcon tubes. Samples were then cooked in a water bath at 80 °C for 30 min. After treatment, the content of the tubes was recovered in a previously weighed crucible. The determination of the parameters was as follows:

- Total losses CL (%): weight of the fluid released after the cooking, expressed in percentage according to the initial sample mass.
- Water loss WL (%): it is the difference between CL and the weight of CL after drying it in oven at 105 °C for 16 h. It is also expressed in percentage depending on the initial mass of the sample.
- Fat Loss (FL in %): it is the difference between CL and WL.

2.2.4. TPA (Texture Profile Analysis)

For texture evaluation, a texturometer (TAPlus LloyD Texture Analyser AMETEK) was used. The type of texture analysis was chosen on the basis according to the properties of the meat product analyzed (Bourne, 1978). To carry out these analyses, 2 cm diameter and 1 cm from the samples were prepared. They were submitted to a double compression up to 50% of the original height by a cylindrical probe of 12 mm diameter and with a speed of 1 mm/s. The measurements were carried out at room temperature. The calculated parameters were:

- Hardness (expressed in N): it sets the force required for the imposed deformation. It is a quantity that matches the maximum height obtained in the first cycle of compression.
- Cohesion (Dimensionless): it is the degree to which the sample deforms before breaking.
- Elasticity (expressed in mm): it is the ability of the sample to regain its original form.
- Adhesion strength (expressed in N/mm): it is the work required to take off the sample from the probe.
- Stiffness (expressed N/mm): it is the ratio between hardness and elasticity.

The analysis was repeated 6 times per sample.

2.2.5. Water activity (aw)

The activity of water was measured using an equipment type NOVASINA Labswift-aw brand capabilities. Simply, a capsule was filled by the sample to half and put in the equipment. The water activity values were displayed after few minutes.

2.2.6. Color measurements

Color parameters measurements, L* (lightness), a* (redness), and b* (yellowness), were made through a solid sample color meter (Color Flex Hunter Associates Laboratory), which was calibrated against a white plate (lightness L* = 93.00, redness a* = 0.3158, and yellowness b* = 0.3323), with an aperture size of 8 mm, and using illuminant D65, a 2° standard observer and an open cone. A cylinder was filled with the sample and the lens of the equipment was placed on the resulting circular surface. L*, a*, and b* were displayed on the screen of the equipment.

2.3. Microbiological analysis

Microbiological analysis was done following the methodology used by Triki et al. (2013). Briefly, 10 g of each sample were placed in a sterile plastic bag with 90 ml of peptone water (0.1%) with 0.85% NaCl. After 2 min in a stomacher blender (Stomacher Colworth 400, Seward, UK), appropriate decimal dilutions were pour-plated (1 ml) on the following media: Plate Count Agar (PCA) (Merck, Germany) for the total viable count (TVC) (30 °C for 72 h); DCA agar - Deoxycholate Citrate Agar (DCA agar) for fecal coliforms (44 °C for 24 to 48 h); Baird Parker Agar (BP agar) for Staphylococcus (37 °C for 24 h); De Man, Rogosa, Sharp Agar (MRS); Violet Red Bile Glucose Agar (VRBG) (Merck, Germany) for Enterobacteriaceae (37 °C for 24 h); and Salmonella Shigella (SS) Agar for Salmonella (37 °C for 24 h). For *Listeria monocytogenes* analysis, the standardized "RAPID'L. mono" detection method was used (NF validation 16140™). Briefly, an enrichment in half Fraser for 24 h ± 2 h at 30 °C ± 1 °C (dilution 1/10) was performed. Then, plating-streaking of 0.1 ml onto "RAPID'L. mono" was achieved. After incubation for 24 h ± 2 h at 37 °C ± 1 °C, the typical colonies were counted. All microbial counts were converted to logarithms of colony-forming units per gram (Log cfu/g).

2.4. Sensory evaluation

Fresh and cooked meat products were assessed by 20 panelists. Various prior training sessions on the product and terminology were conducted to familiarize the panelists with the products. The training sessions were carried out with commercial fresh and cooked meat sausages which were supplied by Chahia industry. Sensory evaluation was made after cooking the fresh meat product by using a microwave during 1 min for each side of the product. This process was used in order to make sure that all the sausages were cooked in same way and that the cooking process would equally affect the sensory evaluation of all formulations. On the other hand, samples were sliced for cooked meat products. Both products were placed on dishes and each formulation carried a 3-digit number. All formulations of every product were evaluated apart at a different moment. The evaluation was conducted by the trained panel. Different parameters were assessed on a scale from 0 to 10 including salty taste (0: not salty, 10: very salty) juiciness (0: not juicy, 10: very juicy), hardness (0: very soft, 10: very hard), texture (0: heterogeneous, 10: homogeneous), color (0: not typical, 10: very typical), and general acceptability (0: dislike, 10: like). For cooked meat sausages, every panelist was given 5 samples from each formulation. In the case of fresh products, the panelist was provided with 3 sausages from each lot. Every product type underwent a separate experiment that included 3 separate sessions where the sensory analysis was repeated.

2.5. Statistical analysis

The statistical analysis of the results was carried out by using the SPSS Statistics program. To determine the effect of the reduction of sodium on the different parameters that were studied, the one-way variance analysis was used (Analysis of variance, ANOVA). The "post hoc" test and the significant difference of Tukey (Honest Significant Difference, HSD) were used for the determination of the differences between the formulations. Treatments (control or reformulated samples), storage time (days), and their interactions were included as fixed effects while replicates were included as a random effect. Means were used to compare differences. The significance level was selected as

Table 1
Mineral composition of the different formulations of fresh and cooked meat products expressed in g/100 g of product.

Formulations	Na	K	Ca
FC	0.98 ± 0.01^{c}	0.34 ± 0.01^{a}	0.034 ± 0.000^{a}
FA	0.70 ± 0.00^{b}	0.38 ± 0.01^{b}	0.047 ± 0.001^{b}
FSM	0.59 ± 0.00^{a}	0.40 ± 0.01^{b}	0.081 ± 0.001^{c}
FN	0.61 ± 0.01^{a}	0.34 ± 0.00^{a}	0.033 ± 0.000^{a}
CC	1.05 ± 0.00^{d}	0.28 ± 0.00^{a}	0.029 ± 0.01^{a}
CA	0.50 ± 0.00^{b}	0.54 ± 0.00^{c}	0.065 ± 0.00^{b}
C80	0.38 ± 0.00^{a}	0.46 ± 0.00^{b}	0.11 ± 0.00^{c}
C50	0.61 ± 0.00^{c}	0.27 ± 0.01^{a}	0.027 ± 0.02^{a}

FC: control fresh meat sausages; FA: fresh meat sausages with Algisalt®; FSM: fresh meat sausages with Salt Mixture (SM); FN: fresh meat sausages without NaCl, Algisalt®, and SM.
CC: control cooked meat sausages; CA: cooked meat sausages with Algisalt®; C80: cooked meat sausages with 80% NaCl substitution by SM; C50: cooked meat sausages with 50% NaCl substitution by SM.
a, b, c, etc. express the significant difference ($P < 0.05$) between different formulations. Statistical analysis have been made separately for each product (fresh (F) and cooked (C) sausages). Within the same product, the statistical analysis was realized separately for each mineral (Na, K, and Ca).

$P < 0.05$. To analyze the sensory data, panelists and sessions were included in a mixed model as fixed effects since the 20 panelists that performed the sensory analysis during the three respective sessions for each product were the same. All analyses and experiments, including the manufacturing processes, were performed in triplicate to guarantee the accuracy of results and the repeatability of the processes.

3. Results and discussion

3.1. Physicochemical properties

3.1.1. Mineral composition

Table 1 presents the results of mineral composition of the different formulations of both products. These results showed a significant reduction $P < 0.05$ of sodium in all reformulated products. However, a lower Na quantity was recorded in FSM and C80 in comparison with FA and CA, respectively. This difference was due to the amount of NaCl present in the AlgySalt®, which corresponds to 25% of the powder. As a matter of fact, a maximum reduction of 39.8% and 63.8% was recorded for FSM and C80 compared to the fresh and cooked control samples, respectively. By taking into account that all added NaCl was substituted by SM in the case of fresh meat products, it is then admitted that the rest of Na comes from the sodium chloride which is already present in the non-meat ingredients and additives, as is the case for cooked meat products. Therefore, it is worth mentioning that every type of product has its own ingredients and additives and hence it has a different concentration of non-added NaCl.

On the other hand, when comparing cooked and fresh sausages' sodium concentrations, it was observed that total NaCl substitution by Algisalt® was more efficient in the cooked products (> 50% reduction) than in the fresh ones (around 30% reduction). This was also the case for the NaCl substitution by SM, which recorded > 60% reduction of Na in the case of C80 compared to 40% reduction in the case of total substitution for FSM. This can be due to the nature and the composition of the products which underwent different manufacture processes.

This study also revealed that the levels of potassium and calcium increased significantly ($P < 0.05$) regarding formulations containing SM or AlgySalt®. This can be explained by the fact that the mixture of salts and AlgySalt® contain high amounts of K, and a moderate quantity of Ca, which would have contributed to the increase of these minerals content in the reformulated products. The presence of these minerals in food is an important factor for human nutrition, given that their consumption would increase the rate of some trace minerals, such as calcium, which exist in minor quantities in natural products. Indeed, the recommended calcium intake is between 1000 and 1300 mg/day. According to Triki et al. (2013), 8 to 10% of this intake is provided by the reformulated products, as it is the case in this study. Indeed, the contribution of Ca per 100 g of product is about 8 to 11%. Moreover, according to the World Health Organization (WHO), the normal daily intake of potassium is about 3500 mg, thus 100 g of reformulated product can contribute with 12 to 14%, which is also in accordance with previous studies (Triki et al., 2013).

Same observations as the ones made for Na, can be made regarding the difference between cooked and meat sausages compositions on potassium. As a matter of fact, greater increase on K was observed in cooked meat products (> 60% increase) compared to fresh ones (around 11% increase) when Algisalt® was used. Moreover, after SM use, 39% increase in K was realized for C80, but only 15% increase in K was observed for FSM. However, even though calcium levels increased by 27% for FA and > 55% for CA, no increase in this mineral was shown when SM was used as a substitute for NaCl. Since Ca quantities were very low compared to Na and K, experimental errors can easily intervene in the results shown in our study. Nevertheless, as mentioned for Na mineral, the differences between the two types of products in K and Ca levels can be due to their dissimilarities when it comes to the nature of their raw ingredients and the way they were processed and conditioned.

3.1.2. pH

Fig. 1 collects pH levels that were recorded for the various formulations of fresh and cooked meat sausages during storage. At the beginning of the experiment, data showed a difference between the types of products. Indeed, for fresh meat sausages, no significant pH differences (around 6.2) ($P > 0.05$) were recorded between formulations. In the case of cooked fresh meat, significant ($P < 0.05$) dissimilarities were observed between “CC and CA” and the rest of the formulations. The AlgySalt® seemed to have maintained the pH of the product despite of the cooking process that it had to undergo, which was not the case for the salt mixture. During the experiment, pH decreased for the fresh product formulations, while it increased for the cooked ones, until the end of the storage. After 5 days of storage, microbiological growth of lactic acid bacteria in fresh meat products (reflected in total viable count in Table 2) tended to acidify the product's matrix. NaCl has a preservative effect as it slowed down flora growth leading to pH maintaining through chilled storage as it has been mentioned in earlier studies (Benkerroum, Daoudi, & Kamal, 2003; Triki et al., 2013). This effect was observed in our study in control samples and the same effect was observed in the products reformulated with SM (Fig. 1a). In this case, the AlgySalt® did not succeed to reduce the pH drop, but it recorded lower pH levels than the ones of FN products.

On the other hand, after 15 days of storage, pH evolution of cooked meat products behaved contrary to the one of fresh meat products (Fig. 1b). As a matter of fact, pH values increased until day 15 for all formulations. It should be noted that for the most of the products this increase marked only < 0.1. Such little increase can be due to experimental analysis variations since the main properties of cooked meat products are the low initial flora and the maintenance of its levels for more than a month. According to Aaslyng et al. (2014), after 24 h of storage, pH of reduced-sodium (50%) cooked products suffered from a slight increase which was not the case for control samples. In other research studies, a substitution of 50% of salt by SM presented no effect on pH (Zanardi et al., 2010).

3.1.3. Cooking loss (water and fat binding properties)

By taking into consideration that fresh meat products are consumed after cooking, these analyses were performed only for the mentioned type of products. Since one of the main effects of NaCl addition in processed foods lies in its binding properties that contribute to the

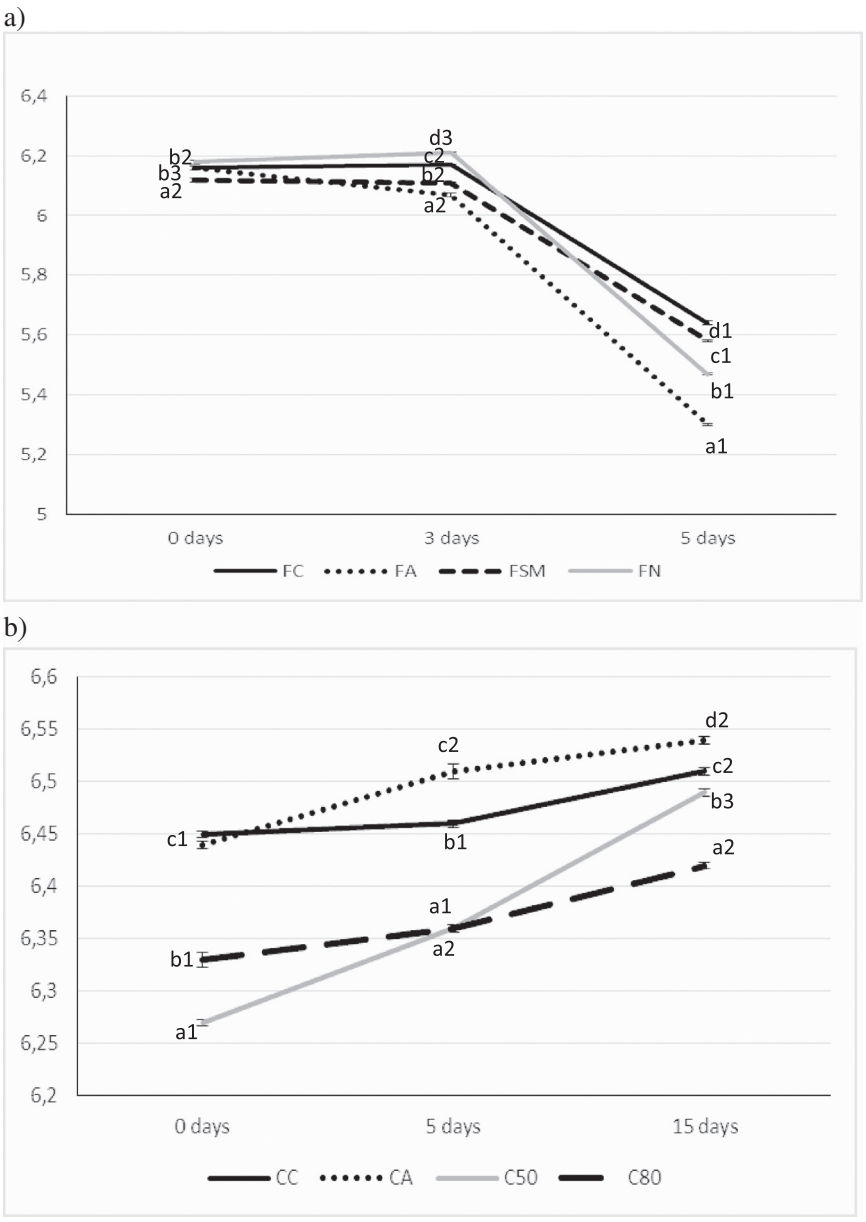


Fig. 1. pH of fresh (a) and cooked (b) meat sausages during chilled storage at 2 °C.
FC: control fresh meat sausages; FA: fresh meat sausages with Algisalt®; FSM: fresh meat sausages with Salt Mixture (SM); FN: fresh meat sausages without NaCl, Algisalt®, and SM.
CC: control cooked meat sausages; CA: cooked meat sausages with Algisalt®; C80: cooked meat sausages with 80% NaCl substitution by SM; C50: cooked meat sausages with 50% NaCl substitution by SM.
a, b, c, etc. express a significant difference ($P < 0.05$) between formulations.
1, 2, 3, etc. express a significant difference ($P < 0.05$) between storage days within the same formulation.

formation of a uniform and consistent meat products matrix by increasing water retention power. This NaCl property also affects product's acceptance by the consumer, which is a very important factor to have a successful reformulation strategy. The most efficient way to study these binding properties is through the total water and fat weight losses after cooking. Results showed in Fig. 2 exhibited no significant differences ($P > 0.05$) between formulations in any of the three losses (total, water, and fat), except for FN which recorded the highest total and water losses ($P < 0.05$) since it contained neither added NaCl nor any type of substituent. Therefore, this study demonstrated that both SM and AlgySalt® have a similar binding property as NaCl. As a matter of fact, sodium chloride interacts with other major components in foods, thereby affecting their textures and the reactions occurring during processing. In particular, salt solubilizes myosin which, upon heating, will form a gel network. It may also stabilize fat particles, in the meat batter (Offer & Trinick, 1983). However, other studies on fresh meat models revealed a significant cooking loss increase between reformulated and control samples (Cofrades, López-López, Ruiz-Capillas, Triki, & Jiménez-Colmenero, 2011; Ruusunen et al., 2005; Triki et al., 2013).

3.1.4. TPA analysis

The results of texture analyses are presented in Fig. 3. Since fresh sausages are presented to the consumers in the market in their fresh form, they are usually evaluated in that state at the time of their acquisition, as well as prior to their cooking and consumption. For that reason TPA of fresh meat products were analyzed before the cooking process. The results presented in Fig. 3a showed that hardness and springiness increased significantly ($P < 0.05$) for samples containing AlgySalt®. On the other hand, the remaining samples were significantly similar ($P < 0.05$) in terms of hardness, whereas FSM samples presented less ($P < 0.05$) amounts than control ones in relation to springiness. However, no significant differences ($P > 0.05$) were observed between control and reformulated products, including the one manufactured with AlgySalt®, for adhesion force, as shown in the Fig. 3a, as well as for chewiness, cohesiveness, viscosity, and elasticity (not presented in Fig. 3). According to Doyle (2008), added salt activates proteins to bind more water molecules in meat. Consequently, this increases tenderness and decreases fluid loss in heat-processed vacuum-packaged products. In the presence of salt, myofibrillar proteins are loosened, which enhances their ability to bind more fat and hence

Table 2
Microbiological analysis of the fresh meat sausages during chilled storage at 2 °C expressed in Log (cfu/g).

Microorganisms		0 days	3 days	5 days
Total viable count	FC	6.34 ± 0.15^{a1}	6.28 ± 0.19^{ab1}	7.04 ± 0.47^{a2}
	FA	6.18 ± 0.48^{a1}	6.20 ± 0.03^{a1}	7.29 ± 0.06^{a2}
	FSM	6.32 ± 0.02^{a1}	6.44 ± 0.16^{b1}	7.22 ± 0.10^{a2}
	FN	6.39 ± 0.01^{a1}	6.27 ± 0.12^{ab1}	7.68 ± 0.12^{b2}
Coliforms	FC	2.97 ± 0.08^{b2}	2.97 ± 0.08^{c2}	2.59 ± 0.11^{a1}
	FA	3.13 ± 0.05^{c2}	2.36 ± 0.04^{a1}	3.05 ± 0.14^{b2}
	FSM	3.09 ± 0.12^{bc3}	2.68 ± 0.08^{b1}	2.93 ± 0.03^{b2}
	FN	2.80 ± 0.02^{a2}	2.28 ± 0.10^{a1}	3.35 ± 0.09^{c3}
Enterobacteriaceae	FC	4.19 ± 0.04^{b2}	3.74 ± 0.17^{a1}	3.88 ± 0.01^{a1}
	FA	4.02 ± 0.02^{a2}	3.91 ± 0.07^{a1}	3.95 ± 0.13^{a12}
	FSM	4.05 ± 0.10^{a1}	4.03 ± 0.16^{a1}	3.90 ± 0.01^{a1}
	FN	3.86 ± 0.20^{a1}	4.04 ± 0.14^{a1}	4.36 ± 0.00^{b2}

FC: control fresh meat sausages; FA: fresh meat sausages with Algisalt®; FSM: fresh meat sausages with Salt Mixture (SM); FN: fresh meat sausages without NaCl, Algisalt®, and SM.
a, b, c, etc. express a significant difference ($P < 0.05$) between formulations.
1, 2, 3, etc. express a significant difference ($P < 0.05$) between storage days within the same formulation.
Within the same product, the statistical analysis was realized separately for each microorganism type.

increase viscosity and form more stable emulsions of comminuted meats. In addition, another characteristic of salt is that it facilitates binding of myosin proteins to each other, improving the texture of processed meats (Man, 2007). Thus, according to our study, the AlgySalt® has an evident hardening effect on the fresh meat products, whereas the mixture of salts seems to have a softening effect on their matrices, which is much more similar to the NaCl effect. The same result was observed for cooked meat products (Fig. 3b), where hardness increased significantly for CA ($P < 0.05$) in comparison with the rest of the formulations, while C80 was significantly softer ($P < 0.05$) than in control samples. This result was also supported by stiffness values. However, unlike the fresh products, the cooked ones presented no significant differences for springiness parameter. It is probably due to the consistent nature of the product, caused by the cooking process that makes it difficult to differentiate springiness variances between formulations.

3.1.5. Water activity (aw)

Water activity is often related to microbial growth since it quantifies the water portion that is available for bacterial proliferation (Christian, 2000; Doyle & Glass, 2010). Therefore, it is important that the reformulation does not increase this parameter. According to our results, apart from FN, no significant differences ($P < 0.05$) were observed in all the formulations after 5 and 15 days of storage for fresh and cooked products respectively whether at the beginning of the experiment or at the end. As a matter of fact, results registered a water activity between 0.95 and 0.96 in all types of products and their formulations combined at any time of the storage process, except for free-salt fresh sausages (0.98). This demonstrates that salt partial and total substitution by SM and AlgySalt® maintained aw amounts, promoting the microbial stability of the final product. Other authors also reported proportions of aw exceeding 0.9 which were also maintained through storage (Zanardi et al., 2010).

3.1.6. Color

According to the data of fresh meat products revealed in Fig. 4, the products reformulated with AlgySalt® presented a significant ($P < 0.05$) lighter aspect than the rest of the formulations at the beginning of the storage and they were very close in terms of lightness. However, at the end of the storage L* parameter increased significantly for all formulations. Indeed, control samples became the lightest and very close to FSM, followed by FA, and finally FN. These results are in total agreement with those reported by Triki et al. (2013), which studied fresh merguez sausages color during refrigerated storage. In their study, L* parameter increased in control and reduced sodium samples. Other authors linked the presence of NaCl in meat products to their luminosity (Toldrá, 2002). The same increasing behavior was observed during storage in a* and b* parameters after 5 days. However, this increase was also detected in all reformulated products in comparison with control ones, from the beginning of the experiment till the end. The increase of such color parameters reflects the effective consequence of adding the substitutes as they play a role in preserving redness and yellowness of the products instead of being decreased through deterioration and oxidation. Other authors reported stable a* and b* parameters amounts (Triki et al., 2013) for control and reduced sodium formulations after 6 days of storage. However after 10 days, the deterioration of products began to cause negative effects on the aforementioned parameters.

On the other hand, for cooked meat products (Fig. 5), no significant difference ($P > 0.05$) was observed for L* parameter at the beginning

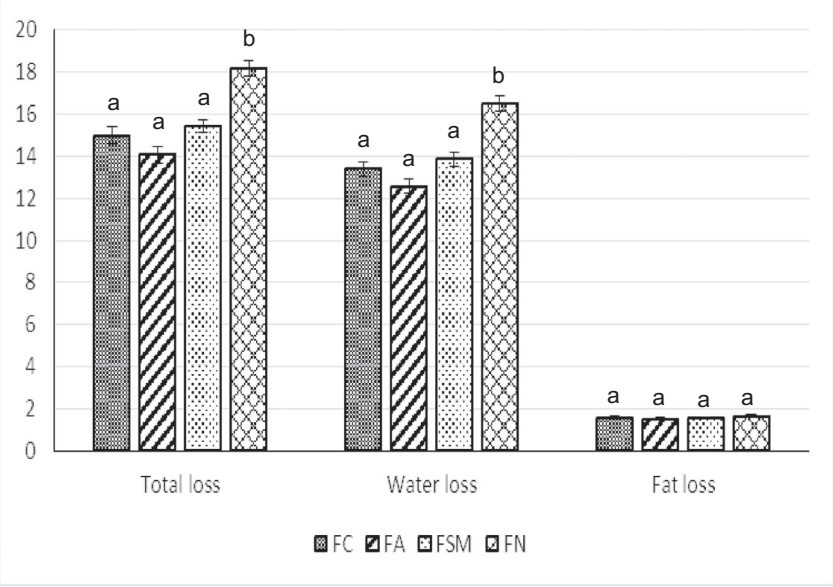


Fig. 2. Cooking loss of fresh meat sausages in g/100 g.
FC: control fresh meat sausages; FA: fresh meat sausages with Algisalt®; FSM: fresh meat sausages with Salt Mixture (SM); FN: fresh meat sausages without NaCl, Algisalt®, and SM.
a, b, c, etc. express a significant difference ($P < 0.05$) between formulations.
Within the same product, the statistical analysis was realized separately for each loss parameter.

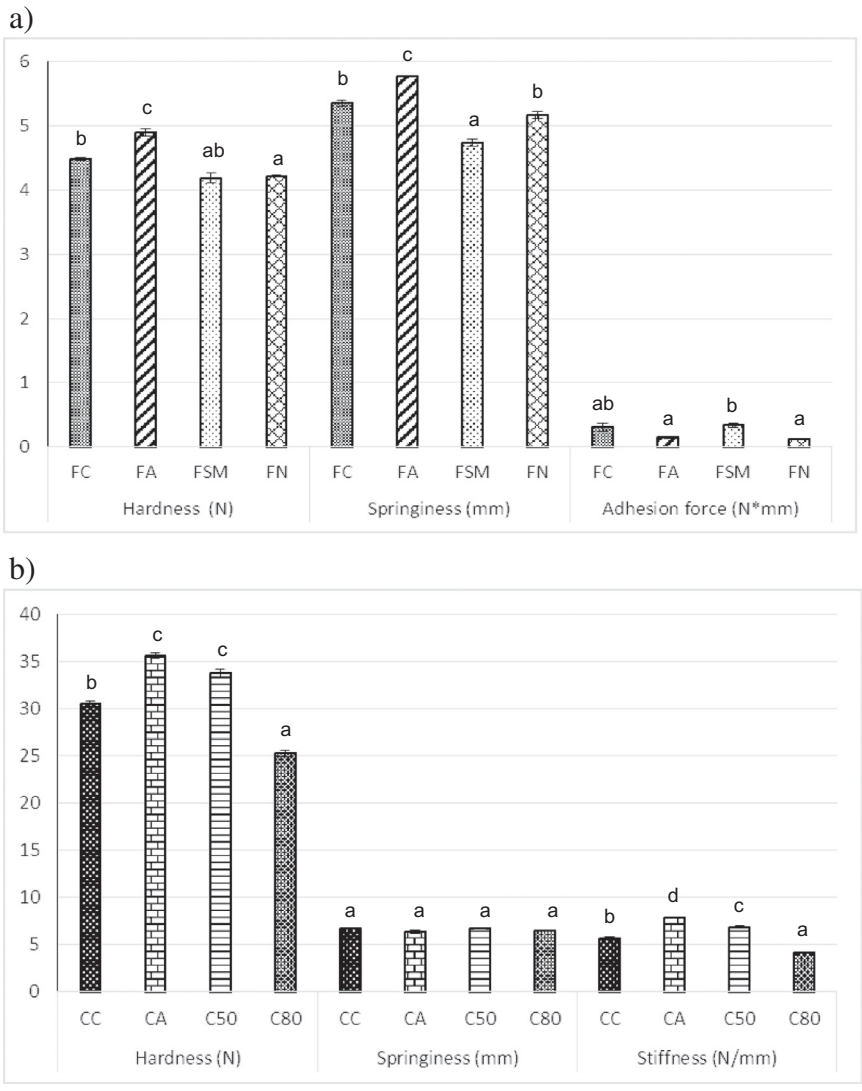


Fig. 3. Most relevant TPA parameters of fresh (a) and cooked (b) meat sausages during chilled storage at 2 °C.
FC: control fresh meat sausages; FA: fresh meat sausages with Algisalt®; FSM: fresh meat sausages with Salt Mixture (SM); FN: fresh meat sausages without NaCl, Algisalt®, and SM.
CC: control cooked meat sausages; CA: cooked meat sausages with Algisalt®; C80: cooked meat sausages with 80% NaCl substitution by SM; C50: cooked meat sausages with 50% NaCl substitution by SM.
a, b, c, etc. express a significant difference ($P < 0.05$) between formulations.
Within the same product, the statistical analysis was realized separately for each TPA parameter.

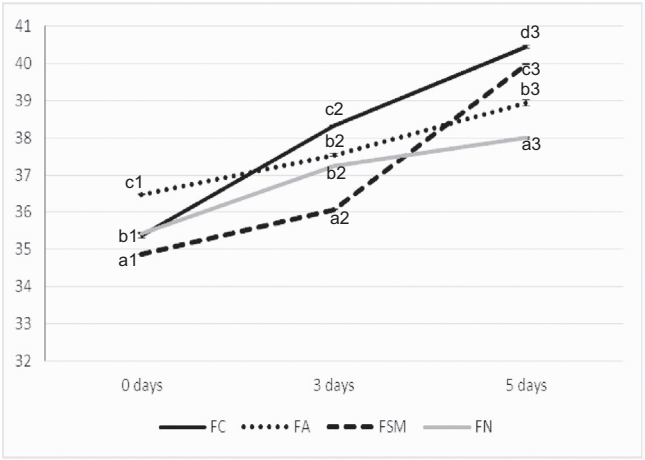
of the storage in all the formulations. However, unlike in the fresh meat product, a significant decrease of lightness was observed in all the other products due to deterioration and oxidation. Control samples underwent the most pronounced decrease, while C80 and C50 experienced the least decrease pronounced one. This demonstrates the positive impact in of SM on maintaining lightness of the final product through storage, which is significantly superior to the AlgySalt® substitute. As it is demonstrated in the case of fresh meat products, SM substitute has also a better color preserving properties than AlgySalt® in cooked meat products. During the experiment, same L* decreasing behavior was observed for a* and b* parameters in all the products, which recorded no significant difference ($P > 0.05$) in terms of redness at the end of the study. However, at the beginning of the storage, products manufactured with SM were redder than the control and CA ones. In the case of b*, control samples started as being the yellowest ones and ended being less yellow than C80, with C50 and CA coming after the aforementioned formulations. The same a* and b* decreasing behaviors were also reported by many previous studies (Du, Hur, & Ahn, 2002; Marcinkowska-Lesiak et al., 2016; Triki et al., 2013).

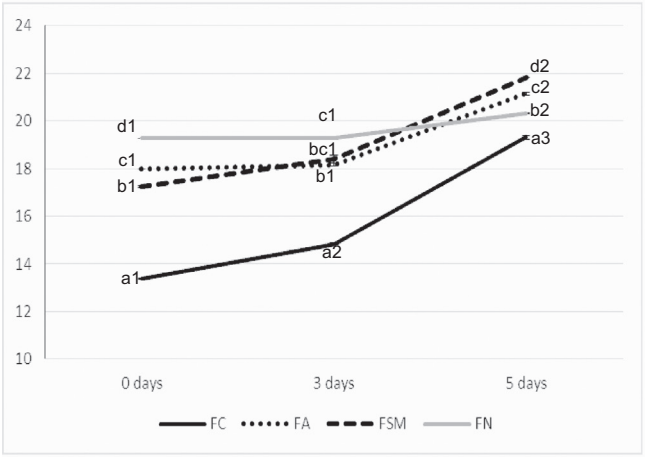
3.2. Microbiology

Six types of microorganisms were counted: total viable count (TVC), thermo-tolerant coliforms, staphylococci, salmonella, *Enterobacteriaceae*, and *Listeria monocytogenes*.

Since *Listeria monocytogenes*, salmonella, and staphylococci were not detected in fresh and cooked meat products during the whole experiment, they were not presented in Tables 2 and 3. For fresh products, results showed that TVC presented no significant differences between reformulated sausages and control ones at the beginning of the storage. This tendency remained until the end of the storage, except for FN sausages, which did not contain NaCl or one of the substitutes. This result demonstrated that SM has the same preserving effect as sodium chloride, which is in agreement with previous studies (Triki et al., 2013). Regardless of their initial levels, which did not present major differences, unlike the TVC counts, coliforms and *Enterobacteriaceae* ones decreased slightly during storage for control and reformulated sausages, with the exception of FN. The same tendency was reported for *Enterobacteriaceae* by other authors (Triki et al., 2013). It is worth noting that microorganisms' counts, including all bacteria types, were always below the legal limits.

On the other hand, results of cooked meat products (Table 3) showed a quasi no initial flora in all formulations since the product underwent a cooking process, which eliminated all types of microorganisms. That is why no significant differences were observed in all products for all types of analyzed bacteria at the beginning of the experiment. However, after 15 days of refrigerated storage, TVC counts reached > 6 logarithmic units in all formulations. While C80 presented the lowest levels of TVC, CA recorded the highest ones, demonstrating the stronger preserving effect of SM in comparison to AlgySalt®.





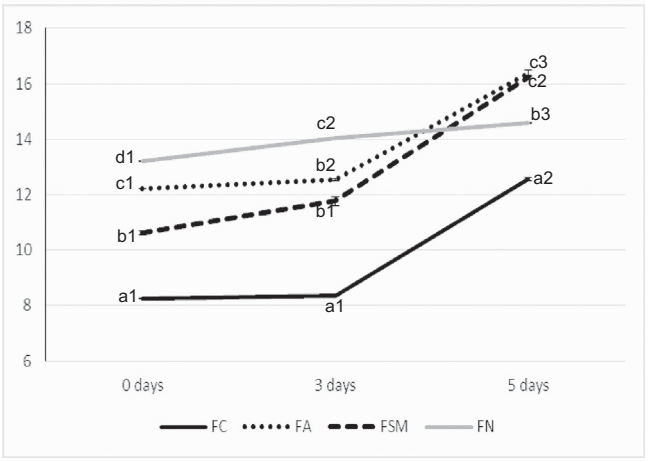
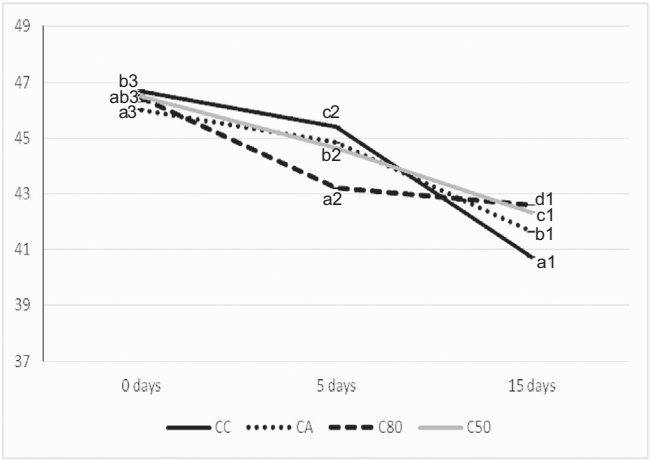
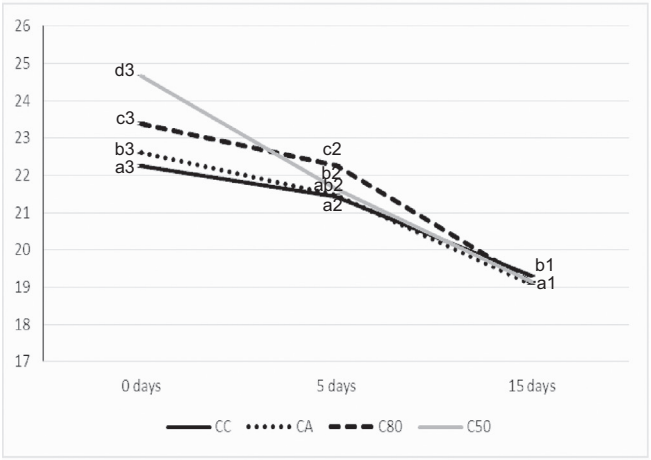


Fig. 4. Color measurements (L*: lightness; a*: red; b*: yellow) of fresh meat sausages during chilled storage at 2 °C.
FC: control fresh meat sausages; FA: fresh meat sausages with Algisalt®; FSM: fresh meat sausages with Salt Mixture (SM); FN: fresh meat sausages without NaCl, Algisalt®, and SM.
a, b, c, etc. express a significant difference ($P < 0.05$) between formulations.
1, 2, 3, etc. express a significant difference ($P < 0.05$) between storage days within the same formulation.

Enterobacteriaceae and coliforms presented no clear growth for all samples during the 15 days storage. These results further demonstrate that the substitutes used during this study have the preservative effect as NaCl, which is in agreement with previous studies (Gimeno et al., 2001).





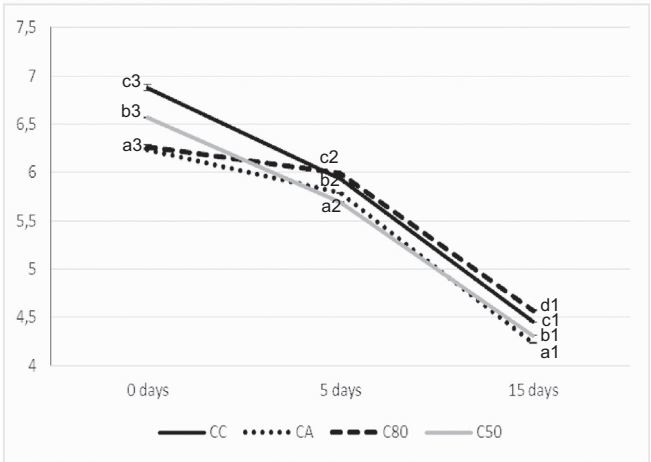


Fig. 5. Color measurements (L*: lightness; a*: red; b*: yellow) of cooked meat sausages during chilled storage at 2 °C.
CC: control cooked meat sausages; CA: cooked meat sausages with Algisalt®; C80: cooked meat sausages with 80% NaCl substitution by Salt Mixture (SM); C50: cooked meat sausages with 50% NaCl substitution by SM.
a, b, c, etc. express a significant difference ($P < 0.05$) between formulations.
1, 2, 3, etc. express a significant difference ($P < 0.05$) between storage days within the same formulation.

3.3. Sensory evaluation

Sensory evaluation for fresh meat products, in this study, was conducted after cooking them for 2 min in the microwave, 1 min on each side of the product. Fig. 6a shows the results of the sensory analysis concerning the fresh meat products. It was observed that FSM and

Table 3
Microbiological analysis of cooked meat sausages during chilled storage at 2 °C in Log (cfu/g).

Microorganisms		0 days	5 days	15 days
Total viable counts	CC	1.00 ± 0.00^{a1}	2.39 ± 0.20^{ab2}	6.32 ± 0.09^{b3}
	CA	1.06 ± 0.21^{a1}	2.58 ± 0.04^{b2}	6.75 ± 0.21^{c3}
	C50	1.00 ± 0.28^{a1}	2.02 ± 0.17^{a2}	6.27 ± 0.02^{b3}
	C80	1.04 ± 0.09^{a1}	2.24 ± 0.09^{a2}	6.01 ± 0.02^{a3}
Coliforms	CC	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}
	CA	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}
	C50	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}
	C80	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}
Enterobacteriaceae	CC	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}	1.69 ± 0.12^{b2}
	CA	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}
	C50	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}	1.47 ± 0.07^{b2}
	C80	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}

CC: control cooked meat sausages; CA: cooked meat sausages with Algisalt®; C80: cooked meat sausages with 80% NaCl substitution by Salt Mixture (SM); C50: cooked meat sausages with 50% NaCl substitution by SM.
a, b, c, etc. express a significant difference ($P < 0.05$) between formulations.
1, 2, 3, etc. express a significant difference ($P < 0.05$) between storage days within the same formulation.
Within the same product, the statistical analysis was realized separately for each microorganism type.

control samples were the mostly similar ones among all the manufactured products, especially in terms of color, salty taste, texture, and juiciness. However, FA samples recorded significantly higher ($P < 0.05$) color, texture, and juiciness punctuations. The behavior of the latter attribute is in accordance with the water cooking loss results found in this study. Indeed, FA presented the lowest levels of water loss between formulations, demonstrating that AlgySalt® indorses strong binding properties among the food constituents and water molecules. This property allowed the product to bind more water after cooking than the rest of the formulations, thus making it juicier when consumed. The diversion between control samples and FA is also noticeable in relation to hardness since AlgySalt® had a significant ($P < 0.05$) hardening effect on the product. This fact is in total agreement with the aforementioned hardening properties enhancement by AlgySalt® (see Section 3.1.4 and Fig. 3a). However, this property does not highlight the textural attributes that AlgySalt® provides to the meat product since it makes FA formulation very different from control samples for consumers. Instead FSM showed very similar sensory properties with control sausages without significantly altering the most important organoleptic aspects of the sausage, which stands for what the consumer is expecting from sodium reduction in a traditional product. On the other hand, FN failed to reach half of the total punctuation (5) in every organoleptic attribute. This formulation was basically manufactured in order to demonstrate the real effect of the substitutes when compared with the control samples. The failure of FN opposed to the successful of the other products made with the substitutes is another evidence that both strategies of reformulations are very effective for this kind of product. Finally and yet surprisingly, FA and FSM received higher punctuations for the hedonic aspect of general acceptability surpassing control samples with > 1 point. A better appreciation of a reformulated product in comparison with its control one is usually attributed to the

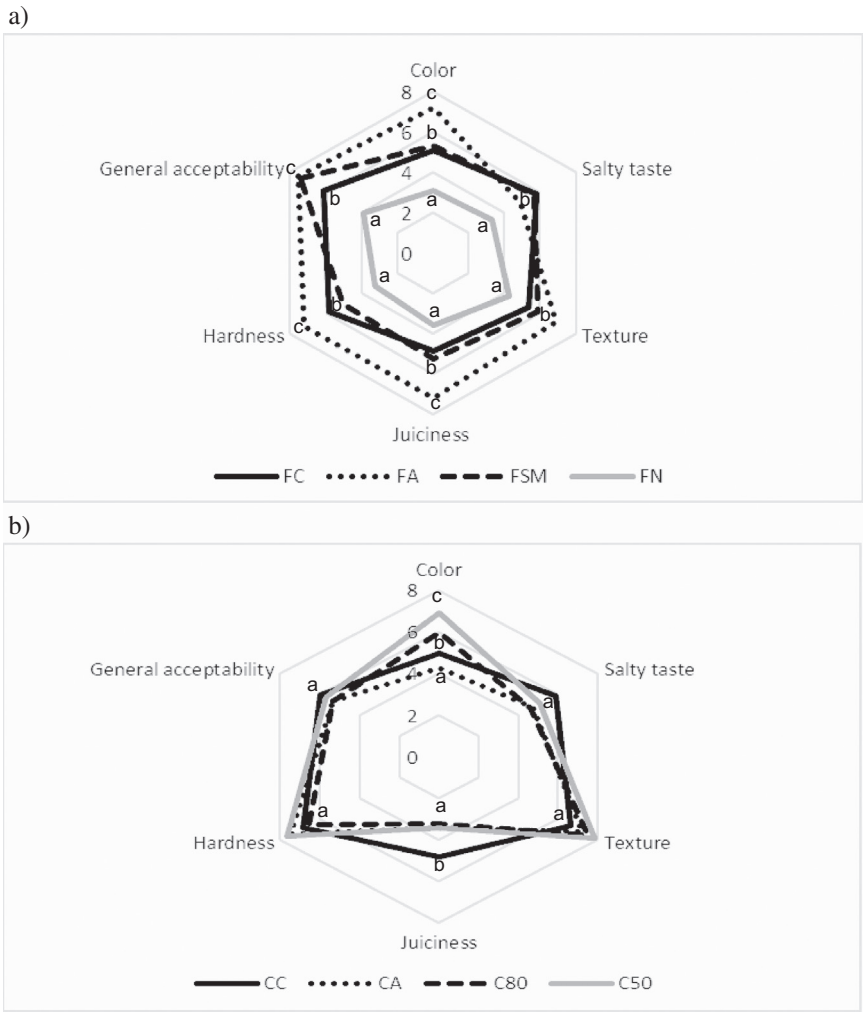


Fig. 6. Sensory analysis of fresh (a) and cooked (b) meat sausages.
FC: control fresh meat sausages; FA: fresh meat sausages with Algisalt®; FSM: fresh meat sausages with Salt Mixture (SM); FN: fresh meat sausages without NaCl, Algisalt®, and SM.
CC: control cooked meat sausages; CA: cooked meat sausages with Algisalt®; C80: cooked meat sausages with 80% NaCl substitution by SM; C50: cooked meat sausages with 50% NaCl substitution by SM.
a, b, c, etc. express a significant difference ($P < 0.05$) between formulations.
Within the same product, the statistical analysis was realized separately for each sensory parameter.

cultural and social backgrounds of the panel (Vabo, 2014). Such an appreciation from a population that is very familiar with the traditional product demonstrates that the substitutes succeeded to highlight certain sensory properties that NaCl failed to enhance.

On the other hand, for cooked meat products, Fig. 6b shows a particular "bell" shape for all formulations with certain differences. Indeed, reformulated products presented the exact form, especially for juiciness and salty taste, which recorded significant ($P < 0.05$) lower levels than control samples. Added to that, color parameter recorded higher levels for C50 and C80 than control ones while CA was significantly lower than CC. At this point, instrumental redness presented the same results as color sensory attributes due to the fact that the panel based its judgment on redness since it is a meat product. These conclusions revealed the color preservation effect of this substitute, which is not the case for AlgySalt®. In contrast with the salty taste and juiciness, texture attributes presented the reformulated products with higher levels compared to CC. This fact was supported by hardness levels as well only in the cases of C50 and CA. For hedonic general acceptability, a very close punctuation was attributed to all formulations with the highest one is for control samples, followed closely by C50. The latter demonstrated very good physicochemical and sensory properties along the study which made it the best reformulation strategy for cooked meat products, followed closely by C80. It is worth noticing that all levels of punctuation for general acceptability exceeded the average level (5).

4. Conclusions

The purpose of this study was to reduce the amount of sodium in fresh and cooked sausages by substituting added NaCl with a mixture of salts (KCl, $CaCl_2$, and $MgCl_2$) and a commercial powder of seaweed extract (AlgySalt®). In order to validate the products and select the best formulations, physicochemical, technological, organoleptic, and microbiological profiles were studied. The most important conclusions were as following: 1) Sodium content of the reformulated products was significantly lower than of the control samples. Potassium and calcium amounts increased significantly ($P < 0.05$) since salt mixture and AlgySalt® were concentrated in K and Ca. 2) In general, samples containing salt mixture (SM) presented better physicochemical and technological properties than the ones formulated with AlgySalt® did. SM sausages were also more similar to control ones in terms of these properties. 3) No clear differences were observed for microbiological count in general, demonstrating that both substitutes have the same preservative effect as NaCl, which is related to the shelf life observed in this experiment. 4) Sensory results showed that reformulated fresh meat products were hedonically better evaluated than control samples; but AlgySalt® based reformulated products were harder than the rest of the formulations. Salt mixture incorporation was the best formulation strategy for fresh and cooked meat products when NaCl was totally substituted for fresh samples (FSM) and partially substituted for cooked meat ones (C50 and C80).

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