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Sodium reduction in "spam-like" product elaborated with mechanically separated tilapia meat

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A R T I C L E I N F O

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A B S T R A C T

This study aimed to evaluate the effect of five substitution levels (0%; 15%; 30%; 45%, and 60%) of sodium chloride (NaCl) by potassium chloride (KCl) on the physical, chemical, technological, bacteriological and sensory qualities of cooked "spam-like" products formulated with mechanically separated tilapia meat (MTM). Substitution of NaCl by KCl increased the weight loss during cooking (between 6.65 and 17.55 g/100 g), fat exudation (between 0.64 and 2.91 g/100 g) and water exudation (between 3.32 and 5.91 g/100 g), and decreased hardness, springiness, cohesiveness and chewiness of the cooked "spam-like" products, representing evidence of the fundamental action of NaCl in the dissolution of myofibril proteins. All the samples were adequate for consumption considering their bacteriological aspects, and the treatments from 15% to 45% substitution showed greater sensory acceptance. The reduction of sodium between 1.05 and 9.2% by substituting NaCl with KCl made it possible to produce a "spam-like" product from MTM, allowing for exploiting this by-product. The product made with 15% substitution of NaCl by KCl was considered the best treatment concerning the technological characteristics, showing less fat and water exudation, less lipid oxidation and higher scores for sensory flavor and global impression.

1. Introduction

Fish, including Nile tilapia (*Oreochromis niloticus*), is highly rich in protein and polyunsaturated fatty acids (PUFAs) such as linoleic acid (ω 6), α-linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (ω 3). These PUFAs are related to relevant health benefits such as reduction of the cardiovascular disease risks, hypotriglyceridemic and anti-inflammatory effects (Cui et al., 2018). The health benefits are one of the main reasons for eating fish, but the lack of practicality, such as bones and the scarcity of ready-to-eat fish products, is one of the principal limitations for its consumption (Carlucci et al., 2015). However, aquaculture's sustainable development is a convincing approach to increase global fish production, providing improvements in human nutrition and food safety. In particular, Nile tilapia is a widely distributed species that could meet the growing demand for fish destined for human food (Baldissera et al., 2020).

Mechanically separated tilapia meat (MTM) is a coproduct of the material leftover's mechanical processing after filleting. Considering that the world tilapia production was 4524.4 tons in 2018 (FAO, 2020), and supposing that 20% of this production was filleted for commercialization and that the yield in MTM after filleting is about 28% (Monteiro et al., 2014), an estimated 253.37 tons of MTM could be available per year for human consumption, a significant volume which could be used in the manufacture of innovative fish products (Secci, Borgogno, Mancini, Paci, & Parisi, 2017). The development of ready-to-eat seafood products from recovered fish by-products could amplify the possibilities of inserting this important protein source in human food, contributing to the companies' sustainability and the

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decrease in environmental impact related to the disposal of solid residues produced during fish processing.

High blood pressure is a great public health problem throughout the world, associated with cardiovascular disorders (Anto et al., 2020). The "World action plan for the prevention and control of non-transmittable diseases (NTD)", established as goals in 2013, a 30% reduction in the mean daily ingestion of sodium by the population, 25% reduction in mortality due to cardiovascular diseases and a 25% reduction in the prevalence of high blood pressure (OPAS, 2017). In addition to impacting the quality of life and individual productivity, NTDs produce high health systems costs. Thus, a decrease in sodium consumption by limiting the amount of salt used in food preparation and the sodium present in industrialized foods is hugely relevant.

Amongst the foods, processed meat products are considered the second greatest item of the diet responsible for salt ingestion (Verma & Banerjee, 2012). However, sodium chloride (NaCl) is of primordial importance as an ingredient of meat products since it acts in the extraction of the myofibrillar proteins (actin and myosin), which are essential for the water and fat holding capacities and the formation of a stable emulsion (Bombrun, Gatellier, Carlier, & Kondjoyan, 2014). From the technological point of view, potassium chloride (KCl) is the best substitute for NaCl due to molecular composition similarity, but its use can be limited due to undesirable sensory attributes (Horita, Messias, Morgano, Hayakawa, & Pollonio, 2014; Stanley, Bower, & Sullivan, 2017). Partial substitution of the NaCl, which interferes with the least possible intensity with the technological, sensory and microbiological parameters, is desirable but still represents an enormous challenge, especially with fish-based products (Gaudett, Pietrasik, & Johnston, 2019). Thus, this study aimed to evaluate the effect of substituting NaCl with KCl chloride on the physical, chemical, technological, bacteriological and sensory qualities of a spam-like product formulated with MTM.

2. Materials and methods

2.1. Material

The MTM was provided by a commercial slaughterhouse in the town of Cachoeiras de Macacu (22°33′58.3″S 42°41′48,2″W), Rio de Janeiro, Brazil. For passion fruit albedo flour (PFF) producing, the skins were first sanitized in a 100 ppm sodium hypochlorite solution for 15 min, the flavedo removed manually with knives, and the resulting albedo blanched in the water at 100 °C for 5 min, dried in an air circulation oven at 60 °C, crushed in an industrial blender and finally ground in a cyclone rotor mill with a 600 μm mesh sieve. The PFF was vacuum packed in low-density polyethylene (LDPE) bags and stored protected from moisture and heat. The PFF was used in the production of tilapia spam to improve the technological aspects of the product such as emulsion stability and weight loss on cooking. The food supplements and additives were acquired from the company FS Commercial (Brand Conatril®) situated in São Carlos, SP, Brazil. Pure analytical (PA) grade reagents of the brand Sigma® were used in the analyses and the culture media used in the microbiological analyses were of the Himedia® brand.

2.2. Processing of the "spam-like' products

An entirely random experimental design with three original repetitions was used for the formulations of the "spam-like" products made from MTM, with five treatments containing different substitution levels of NaCl by KCl (T1: 0%; T2: 15%; T3: 30%; T4: 45% and T5: 60%), which varied but summed up to 1%, and constant ingredients into formulations as shown in Table 1.

The method used to process the experimental products was previously reported by Alves et al. (2016) using a Cutter (Robot Coupe, R602VV, São Caetano do Sul, Brazil). The MTM, variable salt mixture (NaCl + KCl) and ice were first homogenized for 2 min to extract and dissolve the myofibrillar proteins. The remaining ingredients (except the transglutaminase®) were then added and homogenized for a further 2 min. The transglutaminase was added right at the end of the process to promote covalent bonds between the glutamine and lysine. A portion of 400 g of the emulsion was vacuum-packed (Selovac, Jumbo Plus 35, São Paulo, Brazil), cured under refrigeration for 12 h at 4 °C, arranged in ham tins, and cooked under water immersion according to the following scheme: 60 °C for 30 min, 70 °C for 30 min And 80 °C until a temperature of 72 °C was reached at the geometrical center of the food piece. Finally, the pieces were cooled in an ice bath and maintained under refrigeration at 4 °C until analyses.

Table 1
Levels of substitution of sodium chloride (NaCl) by potassium chloride (KCl) in the experimental formulations of spam-like products made with mechanically separated tilapia meat (MTM).

Ingredient (%)[a]	T1 (0%)	T2 (15%)	T3 (30%)	T4 (45%)	T5 (60%)
NaCl	1	0.85	0.70	0.55	0.40
KCl	0	0.15	0.30	0.45	0.60

[a] Besides different levels of NaCl and KCl, all formulations contained 78.45% MTM, 2.5% PFF, 10% ice, 0.25% curing salts, 0.5% California commercial seasoning (Conatril®), 2% emulsifier (Conatril®), 0.25% antioxidant RS/250 (sodium isoascorbate and citric acid) (Conatril®), 0.25 sodium tripolyphosphate (Conatril®), 0.4% flavor intensifier (Conatril®), 2% cassava starch (Conatril®), 1% carrageenan gum, 0.2% garlic powder (Conatril®), 0.2% onion powder (Conatril®), and 1% transglutaminase (Ajinomoto®).

2.3. Physical, chemical and technological characteristics of the experimental products

The experimental products were analyzed for their proximate composition, pH value and water activity according to AOAC (2012), in addition to the instrumental color, total energy value, weight loss on cooking (WLC), emulsion stability, texture profile, lipid oxidation and sodium and potassium contents. The moisture content was determined gravimetrically, drying to constant weight at 105 °C (AOAC nº 925.45), and the total nitrogen content was determined using a Kjeldahl nitrogen analyzer, multiplying by a factor of 6.25 to estimate the crude protein content (AOAC nº 960.52). The ash content of the samples was determined by incineration at 550 °C in a muffler to complete calcination (AOAC nº 923.03), the lipids extracted using petroleum ether in a Soxhlet apparatus (AOAC nº 922.06) and the total carbohydrates calculated by difference (100 – (% moisture + % protein + % lipids + % ash)). The total energy value was calculated using the Atwater conversion factors, multiplying the carbohydrate content by 4, the protein content by 4 and the lipid content by 9 (kcal/g) (Osborne & Voogt, 1978).

The water activity (aw) was determined by direct reading using the equipment (Decagon Devices, CX-2T, Pullman, USA). The pH value was also by direct reading using a digital bench pH-metre calibrated with pH 4.0 and pH 7.0 standard buffer solutions. The instrumental color was determined using a colorimeter (Bankinh Meter Minolta, BC-10, Ramsey, USA) with the color standards of the CIELAB system (Comission Internationale de L'Eclairage), and the results expressed in values for L (lightness), a* (negative = green, positive = red) and b* (positive = yellow, negative = blue). The WLC was determined according to Mcdonnell, Lyng, Arimi, and Allen (2014). The emulsions were packed under vacuum and cooked in a Dubnoff Orbital bath until the central internal temperature reached 72 °C. The WLC was calculated from the difference between the initial and final (after cooking) weights, divided by the initial weight and expressed as a percentage.

The texture profile was determined using a texturometer (Stable Micro Systems, TA. XT2 Plus, Surrey, UK), equipped with a cylindrical 20 mm diameter probe, a distance of 30 mm and pre-test, test and post-test velocities of 1, 1.5 and 10 mm/s, respectively, evaluating the parameters of hardness, cohesiveness, springiness and chewiness. The thickness, height and length of the slices were standardized (20 × 55 × 35 mm), and dimensioned using a digital caliper. The emulsion stability was determined according to Colmenero, Ayo, and Carballo (2005). The emulsion samples (5 g) were centrifuged at 4000×*g* for 5 min, cooked in an orbital Dubnoff bath at 72 °C for 20 min and cooled in an ice bath for 5 min. The exudate was poured into a pre-weighed Petri dish and dried to constant weight in an oven at 105 °C. The results for water exudation (WE) and fat exudation (FE) were expressed concerning the initial sample mass (%).

Lipid oxidation was evaluated by way of the thiobarbituric acid reactive substances (TBARS) according to Yin, Faustman, Riesen, and Williams (1993) and Joseph, Suman, Rentfrow, Li, and Beach (2012). The sample (5 g) was homogenized with 22.5 mL of trichloroacetic acid (11% aqueous solution [w/v]) in an 18 basic Ultra Turrax (IKA, Wilmington, NC, USA) at 11.000×*g* and then centrifuged at 15,000×*g* for 15 min at 4 °C. A 1 mL aliquot of the supernatant was transferred to a tube, and 1 mL of an aqueous 20 mM solution of thiobarbituric acid added. The mixture was vortexed for 5 s, incubated for 20 h in the dark, and the absorbance read at 532 nm. The TBARS were calculated from a standard curve of 1,1,3,3-tetraethoxypropane (concentration range from 0.16 to 820 mg/L) and expressed as mg MDA (malonaldehyde)/kg of meat.

For potassium and sodium determination, the samples were prepared by dry digestion (AOAC, 2012). A portion of homogenized sample (2.5 g) was weighed into a porcelain dish, pre-calcined on a heating plate and then incinerated in a muffle furnace at 450 °C until ash with no black spots was obtained. After dissolving in 1.25 mL concentrated nitric acid, the ash was transferred quantitatively to a 25 mL volumetric flask using purified water. Na and K's readings were made in an emission spectrometer with an inductively coupled plasma source (Varian, ICP OES-Vista MPX, Mulgrave, Australia). Analytical blanks were prepared without the sample and analytical curves in a 5% HNO_3 (v/v) solution. The concentration ranges for the analytical curves were from 0.41 to 410 mg/L for Na and from 0.61 to 610 mg/L for K. All the analyses were carried out in triplicate using three samples for each product (n = 3), except the texture analysis, where six determinations were done per repetition, giving a total of 18 readings per treatment.

2.4. *Bacteriological analyses of the experimental products*

Sample portions (25 g) were homogenized in 225 mL of buffered peptone water and serial dilutions were used for the microbiological analysis. Were tested for Coliforms at 45 °C in Tryptose lauryl Broth (presumptive test) and confirmation in *E. coli* broth, *Salmonella* spp. In Rappaport broth and Selenite Cystine with subsequent streaking on Xylose Lysine Decarboxylase (XLD) agar, coagulase-positive *Staphylococcus* on Bair Parker agar enriched with egg yolk with tellurite, and *Clostridium* sulfite reducing at 46 °C on Tryptose sulfite cycloserine Agar (TSC), according to the methods recommended by the American Public Health Association (APHA, 2001). The microorganisms tested were established according to the microbiological standards for refrigerated or frozen fish-based products cited in Resolution – RDC nº 12, January 12th, 2001 (BRAZIL, 2001), and the analyses carried out in triplicate.

2.5. *Sensory acceptance test*

The acceptance test was carried out in the sensory analysis laboratory with 100 untrained panellists, which were regular consumers of fish products (38 male and 62 female individuals aged between 19 and 55). A nine-point structured hedonic scale was used with the extremes of 1 (disliked immensely) and 9 (liked immensely) to evaluate the attributes of color, flavor, aroma and global aspect (Dutcosky, 2013). Five samples were served to each panelist in a single session, corresponding to the treatments (substitution levels of sodium chloride by potassium chloride), placed on trays and identified by random 3-digit codes. White lighting was used, and the panelists were placed in individual booths. The panelists were instructed to swallow water and wait 30 s between tasting each sample. This study was approved by the Ethics in Research Committee (Report nº 3.032.057).

2.6. *Statistical analysis*

The frequency distribution of the data was normalized and evaluated by the analysis of variance, and the averages smallest significant differences ($p \leq 0.05$) were calculated by Tukey test. Principal components analysis (PCA) correlated the technological and sensory properties of samples, and the Statistica programme (Stasoft Inc., Statistica 11.0, Tulsa, USA) was used for the data analysis and to construct the diagrams.

3. Results and discussion

3.1. *Chemical composition*

The moisture, ash, protein, and carbohydrate contents and the total energy value of the spam-like products were not significantly affected by substituting the NaCl by KCl, contrary to the sodium, potassium, and lipid contents (Table 2). Due to the absence of Brazilian regulations for the spam-like product made from fish flesh and also for the use of MTM, the quality and identity standard for pork, poultry meat or beef spam found in Normative Instruction nº 20 of the Ministry of Agriculture, Livestock and Food Supply (BRAZIL, 2000) was used for comparative purposes, even though they were made from raw materials with different characteristics.

The lipid and moisture contents corroborated with the values described in the legislation mentioned above, which establishes maximum values of 12% and 75%, respectively, for these constituents. All the MTM spam-like experimental products submitted to a reduction in sodium could be classified as low total fat according to Normative Instruction n° 54 (BRAZIL, 2012) since they presented a maximum of 3% total fat. Thus they are all products with health potential, especially for risk groups requiring diets with reduced fat and sodium contents. The significant reduction in the lipid contents as from 30% substitution of NaCl by KCl in the MTM spam-like products can be explained by the effect of sodium on the extraction of the myofibrillar proteins (actin and myosin), necessary for the fat retention capacity and formation of a stable emulsion (Bombrun et al., 2014).

The protein contents determined for the spam-like products (Table 2) were below the limits established by the legislation for pork spam (minimum of 13%), while the carbohydrate contents were higher (maximum of 5%). It can be explained by the fact that the experimental products were produced with MTM, a raw material with a lower protein content (16.98%) than pork (22.6%) (TACO, 2011), and also due to the addition of PFF (5.96% protein) since usually, the addition of fiber and hydrocolloid sources to meat products can decrease the protein contents and increase the carbohydrate contents in the final product (Choi et al., 2013).

According to Brazilian legislation, the products studied (Table 2) can be classified as median energy value foods (BRAZIL, 2012). The results found in the present study referring to the lipid, carbohydrate, and total energy value contents agree with the values reported by Bessa et al. (2016). In a study with Frankfurter-type sausages made with MTM with 50% of the NaCl substituted by KCl and the addition of inulin, these authors observed a tendency to decrease the lipid content and increase the carbohydrates content as a fat substitute due to the addition of inulin, a tendency also observed in the present study due to the use of PFF.

As expected, there was a significant decrease ($p < 0.05$) in the sodium levels (from 885.33 to 782.66 mg/100 g) and an increase in the potassium levels (from 219.99 to 437.00 mg/100 g) as a result of the increase in substitution of the salt in the spam-like products (Table 2). Such products are generally classified as high sodium content products

Table 2
Proximate composition, sodium and potassium contents and energy value of the spam-like products made with mechanically separated tilapia meat (MTM) with different levels of substitution of sodium chloride (NaCl) by chloride (KCl).

Parameter	Treatments - Level of substitution of NaCl by KCl (%)[1]				
	T1 (0%)	T2 (15%)	T3 (30%)	T4 (45%)	T5 (60%)
Moisture[2]	$66.24^{A} \pm 0.82$	$65.70^{A} \pm 0.43$	$64.80^{A\pm}1.67$	$65.46^{A} \pm 0.27$	$65.90^{A} \pm 0.81$
Ashes[2]	$9.50^{A} \pm 0.40$	$9.35^{A} \pm 0.25$	$9.74^{A} \pm 0.76$	$9.23^{A} \pm 0.75$	$9.75^{A} \pm 0.33$
Protein[2]	$10.07^{A} \pm 0.45$	$10.06^{A} \pm 0.31$	$9.95^{A} \pm 0.24$	$9.94^{A} \pm 0.48$	$10.03^{A} \pm 0.52$
Lipids[2]	$2.73^{A} \pm 0.07$	$2.45^{AB} \pm 0.28$	$2.22^{B} \pm 0.10$	$2.10^{B} \pm 0.05$	$2.14^{B} \pm 0.24$
Carbohydrates[2]	$11.46^{A} \pm 0.95$	$12.44^{A} \pm 0.72$	$13.29^{A} \pm 1.13$	$13.27^{A} \pm 0.58$	$12.18^{A} \pm 0.65$
Sodium[3]	$885.3^{A} \pm 2.2$	$876.0^{B} \pm 0.7$	$814.0^{C} \pm 1.3$	$804.0^{D} \pm 1.3$	$782.7^{E} \pm 2.9$
Potassium[3]	$219.0^{E} \pm 2.0$	$266.3^{D} \pm 0.9$	$305.3^{C} \pm 3.1$	$353.3^{B} \pm 1.1$	$437.0^{A} \pm 1.1$
TEV[4]	$110.69^{A\pm}$ 3.29	$112.05^{A\pm}2.88$	$112.94^{A\pm}$ 4.99	$111.74^{A\pm}$ 2.25	$108.10^{A\pm}$ 1.55

[1]Results expressed as the mean ± standard deviation (n = 3). Means followed by the same letter in the same line are not statistically different by Tukey's test ($p<0.05$); [2](%); [3](mg/100 g);[4]Total energy value (kcal/g).

for presenting values above 80 mg/100 g of this mineral (BRAZIL, 2012), but it is worth highlighting that it is not only sodium chloride that is responsible for the increase in this mineral but also other ingredients such as sodium tripolyphosphate, curing salts, flavor enhancers, antioxidants and seasoning. Comparing T3, T4, and T5 with T1, reductions in the Na content between 8.05; 9.2 and 11.6% were noted in the spam-like products, so the treatments caused a significant decrease in the sodium levels when compared to the control.

The maximum limit recommended for sodium by the World Health Organization (WHO) is 2 g per day for adults and children, which corresponds to the consumption of 5 g per day of sodium chloride (WHO, 2012a). Considering the maximum recommended ingestion of Na as the basis, one could consume a maximum of 229, 246, 250 and 256 g per day of the MTM products for treatments T2 to T5, respectively. Although the experimental products could not be classified as having a reduced sodium content, the results encountered could contribute to governmental initiatives and expectations for the reduction of the ingestion of this mineral in meat products, aiming to meet the maximum limit recommended by WHO, and contributing to the health of consumers of spam-like products, more specifically of spam itself.

Regarding the potassium content of the experimental products, it increased in a level-dependent manner when compared with T1 (Table 2). The minimal daily ingestion of potassium recommended by WHO for adults is 3.5 g to decrease the blood pressure and the risk of CVAs and coronary diseases (WHO, 2012b). Thus the ingestion of 100 g of the MTM product could provide from 0.22 to 0.43 g potassium, contributing to an increase in potassium ingestion from the diet in agreement with the recommended daily minimum.

3.2. Physicochemical and technological characterizations

The replacement of NaCl by KCl affected the physicochemical and technological properties of the spam-like products (Table 3). In general, increases in the substitution of NaCl by KCl significantly increased ($p < 0.05$) the weight loss on cooking (WLC), fat exudation (FE), water exudation (WE) and lipid oxidation (TBARS), and significantly reduced ($p < 0.05$) luminosity (L*), hardness, springiness and chewiness, but did not affect ($p > 0.05$) the pH value, water activity (aw), chroma a* and chroma b*.

The values for TBARS observed in the present study varied from 0.43 to 0.68 mg MDA/kg of meat, increasing with the substitution level of NaCl by KCl (Table 3). Lipid oxidation, known as 'warmed over flavor' is the leading cause of change in cooked meat foods, due in part to high temperatures. Protein denaturation and the partial destruction of muscle structures allow for the interaction between unsaturated lipid molecules and pro-oxidant elements. During cooking, the iron is released from haem pigments such as myoglobin, a well-known pro-oxidant agent of lipid hydro-peroxides. The homolytic bond (-O-O-) is cleaved, forming two free radicals for each peroxide, thus accelerating lipid autoxidation (Monahan, 2000). MTM favors oxidative processes due to the presence of polyunsaturated fatty acids (Lombardo & Chicco, 2006), as also the cooking process to which the experimental products were submitted at temperatures between 60 and 80 °C.

The reduction in sodium chloride presented a directly proportional pro-oxidant effect in the experimental products. The predominance of polyunsaturated fatty acids in MTM, allied to the substitution of NaCl could have interfered in the TBARS contents in the present study. Although sodium chloride has a proven pro-oxidant effect, some authors have reported little or no effect of the NaCl concentration on lipid

Table 3
Physical, physicochemical and technological characteristics of the spam-like products made with mechanically separated tilapia meat (MTM) with different levels of substitution of sodium chloride (NaCl) by potassium chloride (KCl).

Parameter	Treatment (level of substitution of NaCl by KCl)[1]				
	T1 (0%)	T2 (15%)	T3 (30%)	T4 (45%)	T5 (60%)
Fat oxidation[2]	$0.43^{E} \pm 0.07$	$0.54^{D} \pm 0.01$	$0.58^{C} \pm 0.01$	$0.62^{B} \pm 0.02$	$0.68^{A} \pm 0.02$
WLC[3]	$6.65^{E} \pm 0.22$	$7.83^{D} \pm 0.08$	$10.65^{C} \pm 0.17$	$13.22^{B} \pm 0.09$	$17.55^{A} \pm 0.17$
Fat extruded[3]	$0.64^{E} \pm 0.01$	$0.95^{D} \pm 0.01$	$1.13^{C} \pm 0.06$	$1.86^{B} \pm 0.02$	$2.91^{A} \pm 0.01$
Waterextruded[3]	$3.32^{D} \pm 0.21$	$3.63^{CD} \pm 0.04$	$3.92^{C} \pm 0.01$	$4.73^{B} \pm 0.20$	$5.91^{A} \pm 0.03$
Hardness[5]	$33.53^{A} \pm 2.35$	$32.03^{AB} \pm 2.23$	$31.50^{B} \pm 1.06$	$29.40^{C} \pm 3.29$	$28.00^{C} \pm 2.57$
Springness[6]	$0.89^{AB} \pm 0.02$	$0.90^{A} \pm 0.02$	$0.88^{AB} \pm 0.03$	$0.86^{BC} \pm 0.03$	$0.82^{C} \pm 0.01$
Cohesiviness[6]	$0.55^{A} \pm 0.07$	$0.54^{A} \pm 0.07$	$0.42^{B} \pm 0.06$	$0.41^{B} \pm 0.07$	$0.34^{B} \pm 0.05$
Chewiness[7]	$15.34^{A} \pm 2.40$	$15.35^{A} \pm 2.67$	$14.76^{A} \pm 4.74$	$11.51^{AB} \pm 3.47$	$7.66^{B} \pm 1.59$
pH[4]	$6.70^{A} \pm 0.04$	$6.69^{A} \pm 0.01$	$6.70^{A} \pm 0.07$	$6.65^{A} \pm 0.06$	$6.74^{A} \pm 0.02$
aw[4]	$0.97^{A} \pm 0.01$	$0.97^{A} \pm 0.05$	$0.97^{A} \pm 0.02$	$0.97^{A} \pm 0.01$	$0.97^{A} \pm 0.02$
Luminosity[4]	$57.15^{AB} \pm 1.13$	$57.68^{A} \pm 0.40$	$55.93^{BC} \pm 0.50$	$55.48^{BC} \pm 0.50$	$54.81^{C} \pm 0.40$
Chroma a*[4]	$13.14^{A} \pm 0.72$	$13.30^{A} \pm 0.73$	$14.22^{A} \pm 0.57$	$14.28^{A} \pm 0.56$	$14.27^{A} \pm 0.24$
Chroma b*[4]	$16.84^{A} \pm 1.17$	$18.27^{A} \pm 1.04$	$16.88^{A} \pm 0.36$	$16.77^{A} \pm 0.65$	$17.33^{A} \pm 0.44$

[1]Results expressed as the mean ± standard deviation (n = 3). Means followed by the same letter in the same line are not statistically different by Tukey's test ($p<0.05$); [2]mg MDA/kg; [3]%; [4]dimensionless; [5]Results expressed in Newton (N); [6]Results expressed as proportion; [7]Results expressed in N x mm.

oxidation. Sarraga and García-Regueiro (1998) reported that an increase in NaCl concentration resulted in lower TBARS levels and suggested a possible antioxidant effect of the salt in meat and meat products, in agreement with the present study and with the results obtained by Andrés, Cava, Ventanas, Muriel, and Ruiz (2004), the 1% of NaCl used in the control formulation possibly being responsible for the reported effect. The salt content exerts an influence on the lipid oxidation of meat products, and a relationship exists between the raw material used and the processing conditions (Wang, Jin, Zhang, Ahn, & Zhang, 2012), justifying the results found in the present study.

The maximum limits permitted for mg MDA/kg of meat can vary between 0.5 and 0.6 (Choi et al., 2010; Fang, Lin, Warner, & Ha, 2018). Considering a limit of 0.6 mg MDA/kg of meat, substitution levels of 45 and 60% of the NaCl by KCl resulted in values above the referenced authors' limit. However, Brunton, Cronin, Monahan, and Durcan (2000) concluded that TBARS values as from 1.5 mg MDA/kg of meat could cause the formation of off-flavors in cooked turkey breast, which has a predominance of polyunsaturated fatty acids, as in MTM, showing satisfactory results for this parameter in the present study, when compared to the values reported by the above-cited authors. Guo et al. (2019) observed TBARS values below 0.6 mg MDA/kg at the end of 2 days salting, however TBARS values above 0.6 mg MDA/kg during 4, 6, and 8 days of salting process in dry-salted grass carp prepared with 51.3% of substitution of NaCl by KCl. Ganie, Kumar, Dua, and Raja (2017) evaluated the overall quality during refrigerated storage of low sodium fish (*Pangasius pangasius*) balls from substitution of 50% of NaCl by 40% KCl, 30% citric acid and 30% sucrose. These authors reported TBARS values within the referenced authors' limit (0.6 mg MDA/kg) up to 7 days under refrigeration, which was exceeded on days 14 (0.64 mg MDA/kg) and 21 (0.71 mg MDA/kg).

Exudation during the cooking process represents a relevant change in meat products with high moisture content, resulting in economic losses due to the loss in weight, changes in the nutritional value due to the release of soluble vitamins and amino acids, and causing prejudicial effects to the texture and succulence of the final product (Bochi, Weber, Ribeiro, Victório, & Emanuelli, 2008). The WLC of the MTM spam-like products varied between 6.65 and 17.55%, the FE between 0.64 and 2.91% and the WE between 3.32 and 5.91%, increasing with the level of substitution of NaCl by KCl (Table 3).

The methodologies employed to obtain the emulsified products require solubilization and extraction of the myofibril proteins (actin and myosin) with NaCl to form a protein gel. Decreasing the percent of NaCl induces a reduction in the amount of protein solubilized and extracted, which affects the binding capacity of the resulting emulsion (Ramírez, Uresti, Velazquez, & Vázquez, 2011). Thus, the WLC, FE and WE were significantly affected by the substitution levels of NaCl by KCl, causing a directly proportional increase in the water and fat exudations and WLC.

The levels of sodium chloride used in processed meat products have a considerable influence on the meat emulsions' bonding capacity. The amount of solubilized protein is directly proportional to the system's ionic strength, potentiating the interactions of the polypeptide chains during heating, resulting in the formation of a more stable gel matrix, which results in less water and fat exudation (Carballo, Mota, Barreto, & Jiménez Colmenero, 1995). The ionic strength is exerted by the presence of sodium chloride, which leads to solubilization of the myofibril proteins, forming a viscous exudate on the surface of the emulsified product, promoting retention of the free water and fat during cooking (Terrell, 1983). The results obtained agree with the data determined by Horita et al. (2014), who reported a reduction in the emulsion's stability directly proportional to sodium chloride substitution by potassium and magnesium chlorides, resulting in more significant fat and water losses.

Although the enzyme transglutaminase, carrageenan gum, and cassava starch were used in the present study, aiming to improve gel formation in the spam-like products, the products' technological properties and texture profiles were affected by the levels of substitution of the NaCl by KCl. As a result, the values for hardness, springiness, cohesiveness and chewiness decreased with an increase in NaCl substitution by KCl (Table 3).

As described by Carballo, Ayo, and Jiménez Colmenero (2006), transglutaminase requires the addition of NaCl to improve the functional and textural properties of restructured foods. The authors concluded that sodium salts were necessary to promote the protein-water interactions required to obtain adequate water-holding properties in cooked products with added transglutaminase. The reduction in NaCl decreased the ionic strength and hence reduced the levels of protein solubilized as the substrate for the action of the enzyme, thus reducing reticulation of the myofibril proteins. This enzyme's action can be explained based on the increase in swelling power and water absorption allied to its jellifying capacity (Ruiz-Carrascal & Regenstein, 2002), and the best textural properties were obtained with 2% NaCl (Ramírez & Cava, 2007). However, as the objective was to reduce the Na content, the maximum NaCl concentration used in the present study was 1%, which could have contributed to the formation of emulsions with limited stability as the level of substitution of the NaCl by KCl increased. In this scenario, there is a decreased solubilization of the myofibril proteins and reduced occurrence of reticulation due to enzyme action, besides the presence of MTM as the raw material, which has a muscle arrangement already altered by its production process.

Hardness is measured by the maximum force required to compress the sample between the molar teeth; springiness expresses the height the sample returns to after being submitted to compression, returning to its original format; cohesiveness represents the extension to which the sample can be deformed before rupture; and chewiness the work required to chew a solid sample until it disintegrates sufficiently to be swallowed (Bourne, 1978). Thus the results obtained for hardness (Table 3) corroborated with the findings of Pietrasik, Gaudette, and Klassen (2015), who found that the reduction in salt content resulted in the production of Frankfurter sausages that were less hard than those of the control. A tendency for decreased hardness was observed by Paula et al. (2019) when the NaCl content of cooked hams was reduced.

The values for pH and aw for all the treatments studied (Table 3) were considered adequate and typical for the ranges recommended for cooked ham and fish flesh: pH between 6.0 and 7.0 and aw between 0.93 and 0.97 (Damodaran, Fennema, & Parkin, 2010; Jay, 2005; Kachele, Zhang, Gao, & Adhikari, 2017). These values are in the ranges that favor the growth of pathogenic and deteriorative bacteria, and hence the products had to be stored under controlled refrigerated conditions using adequate packaging material. The aw is a relevant quality control indicator for foods since the multiplication of bacteria is favored when the aw is above 0.9. A pH value close to neutrality favors pathogenic bacteria's growth, being an essential intrinsic factor of foods to be considered in developing products and the conservation methods to be employed.

Color is an important quality parameter for consumers when deciding whether to buy meat products, and color changes can decrease their acceptability (Cáceres, García, & Selgas, 2008). In general, the values for L* (57.68–54.81) decreased with an increase in the substitution of NaCl by KCl, indicating a slight darkening of the samples (Fig. 1). A result in agreement with the reports of Barretto et al. (2020), where the incorporation of 0.5% KCl in the formulation of cooked ham reduced the value for L*, and of Horita et al. (2014), who observed a significant reduction in the value for L* of Frankfurter sausages on adding 0.6% KCl. This effect was not verified for the chromaticity coordinates, where the values did not vary significantly for b* (yellow axis) or a* (red axis). Pietrasik and Gaudette (2014) did not detect any effect on the values for a* with a decrease in the salt contents of spam samples, in agreement with the present study results.

3.3. Microbiological profile

The MTM spam-like products were analyzed bacteriologically to verify the effect of the production stages on the final product quality and

Fig. 1. Experimental products made with different levels of substitution of sodium chloride (NaCl) by potassium chloride (KCl).

assure safety for consumers in the sensory evaluation. The Brazilian National Health Surveillance Agency (BRAZIL, 2001) establishes a maximum count of 10^3 coliforms at 45 °C/g, 3 × 10^3 *Staphylococcus* positive coagulase/g, 5 × 10^2 *Clostridium* sulfite reducer at 46 °C, and absence of *Salmonella* spp. In 25 g for cooked meat products. In our study, regardless of the level of substitution of NaCl by KCl, all MTM spam-like products were in line with the legal requirements, demonstrating a satisfactory sanitary quality (Table 4). It indicates the efficiency of the heat treatment applied during cooking of the products, and the satisfactory sanitary conditions adopted during their elaboration. Other previous studies (Ganie et al., 2017; Orel et al., 2020) also obtained a satisfactory microbiological quality after use of different levels of salt replacers in cooked meat products.

3.4. Sensory acceptance

The substitution level of NaCl by KCl did not affect the appearance, aroma, or color of the MTM spam-like products (Table 5). All the treatments were considered acceptable from the sensory point of view. T2 and T3 were awarded the highest mean scores for flavor and T2 for global impression, but they only differed from T5, which received a significantly lower mean score ($p < 0.05$). For the parameters of appearance, aroma, flavor and global impression, the means obtained corresponded to between "neither liked nor disliked" and "liked slightly" on the hedonic scale, whereas for the color, they were between "disliked slightly" and "neither liked nor disliked". However, the experimental products were statistically more accepted with up to 45% substitution of NaCl by KCl. For meat hamburgers (beef and pork), up to

Table 4
Bacteriological profiles of the spam-like products made with different levels of substitution of sodium chloride (NaCl) by potassium chloride (KCl).

Bacterium	Treatment (level of substitution of NaCl by KCl)				
	T1 (0%)	T2 (15%)	T3 (30%)	T4 (45%)	T5 (60%)
Coliforms at 45 °C[b]	<30	<30	<30	<30	<30
Staphylococcus positive coagulase[a]	<10	<10	<10	<10	<10
Clostridium sulfite reducer at 46 °C[a]	<10	<10	<10	<10	<10
Salmonella spp.[c]	absent	absent	absent	absent	absent

[a] CFU/g.
[b] MPN/g.
[c] Absence in 25 g.

Table 5
Mean scores obtained in the acceptance test of the spam-like products made with mechanically separated tilapia meat (MTM) with different levels of substitution of sodium chloride (NaCl) by potassium chloride (KCl).

Attribute	Treatment (level of substitution of NaCl by KCl)					p value/ standard error
	T1 (0%)	T2 (15%)	T3 (30%)	T4 (45%)	T5 (60%)	
Appearance[1]	5.29^{A}	5.31^{A}	5.10^{A}	5.34^{A}	5.18^{A}	0.55/0.11
Aroma	5.36^{A}	5.48^{A}	5.26^{A}	5.22^{A}	5.15^{A}	0.67/0.16
Flavor	6.03^{AB}	6.50^{A}	6.34^{A}	6.13^{AB}	5.60^{B}	0.006/0.17
Color	4.98^{A}	5.17^{A}	5.05^{A}	5.15^{A}	5.15^{A}	0.70/0.11
Global impression	5.70^{AB}	6.17^{A}	5.86^{AB}	5.89^{AB}	5.53^{B}	0.01/0.13

[1]Means followed by the same letter in the same line are not statistically different by Tukey's test ($p < 0.05$).

50% of the NaCl could be substituted by KCl without negatively influencing the products' sensory acceptance (Lilic et al., 2015), a value close to that obtained in the present study for the MTM spam-like product. The addition of KCl to meat products is limited by developing a bitter, metallic taste. Thus the use of ingredients to minimize this effect should be taken into consideration in future studies.

3.5. Principal component analysis

The principal components analysis (PCA) of the physical, chemical and technological properties and sensory attributes of the MTM spam-like products with different levels of substitution of the sodium chloride (NaCl) by potassium chloride (KCl), showed that two principal components, PC1 (56.1%) and PC2 (19.28%), explained 76.09% of the total variance (Fig. 2). The substitution level of NaCl by KCl in the formulations showed a positive correlation with the lipid and water contents, WLC, FE, WE, a*, lipid oxidation and potassium content; and negative correlation with L*, hardness, springiness, cohesiveness, chewiness and sodium content. Thus, NaCl's substitution by KCl caused increases in the lipid and water contents, WLC, FE, WE, a*, lipid oxidation and potassium content; and reductions in L*, hardness, springiness, cohesiveness, chewiness and sodium content.

According to the Score Plot (Fig. 2), there was a distinction between the four groups (T1, T2, T3 and T4) and T5. Thus the formulations with 30% and 45% of NaCl substitution by KCl showed similar physical, chemical, technological and sensory characteristics between them but were different from the others, which differed among them. The ash and protein contents, b*, appearance and color had little influence on the principal components, which can be seen from the coefficients of these variables and the sizes of the vectors in Fig. 2 (Correlation circle).

The potassium content showed a negative correlation with the sodium content due to the substitution of NaCl by KCl; that is, as the KCl concentration increased, so the sodium concentration decreased in the spam-like products, which was to be expected in the present study. Smaller angles between the variables indicated a positive correlation between the parameters. Thus, the moisture content and water activity showed a positive correlation with each other as well as springiness with chewiness; flavor with global impression; pH with the lipid content; FE with WE; WLC with the potassium content; and hardness and cohesiveness with L*.

The Score Plot (Fig. 2) shows that the formulations with 0% and 15% of KCL suffered more influence from the variables of moisture content, water activity, lightness, hardness, springiness, cohesiveness, chewiness, aroma, flavor and global impression. However, the formulation with 60% of KCl was more influenced by the lipid content, WLC, FE, WE, lipid oxidation and potassium content. Analyzing the Score Plot, one can highlight the fact that T2 showed characteristics similar to those of the experimental standard, and considering the correlations, it could be considered the best treatment, mainly for the technological characteristics, with the least FE and WE, least lipid oxidation and highest scores

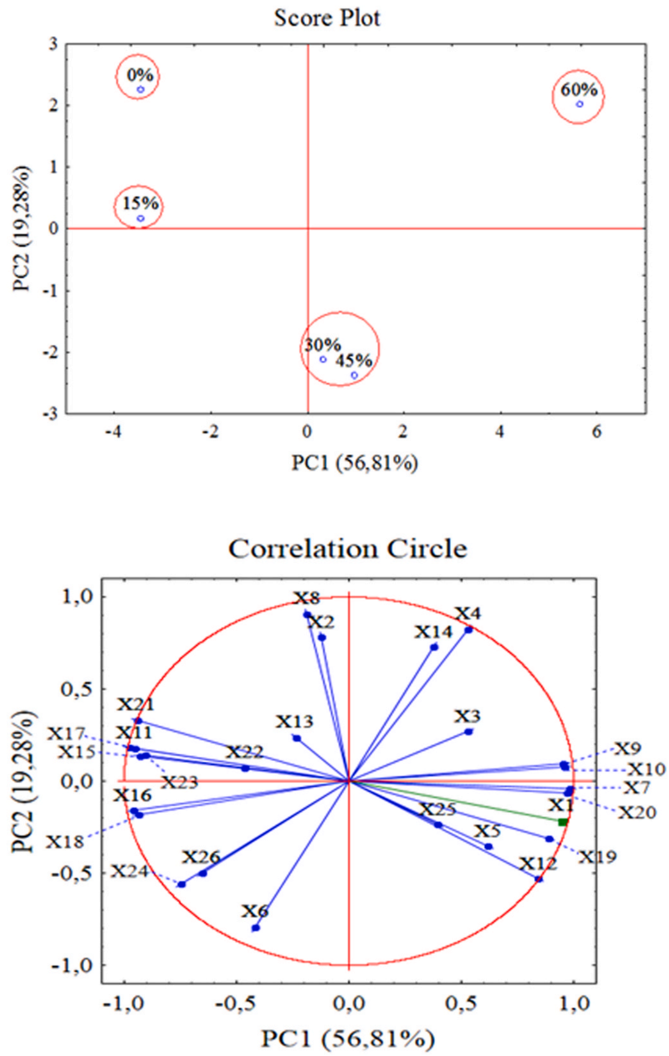

Fig. 2. Principal components analysis (PCA) of the spam-like products made with mechanically separated tilapia meat (MTM) with different levels of substitution of sodium chloride (NaCl) by potassium chloride (KCl).
Where: X1 = level of substitution of NaCl by KCl; X2 = moisture content, X3 = ash, X4 = lipid, X5 = protein, X6 = carbohydrate, X7 = weight loss on cooking, X8 = water activity, X9 = fat exudation, X10 = water exudation, X11 = lightness, X12 = a*, X13 = b*, X14 = pH value, X15 = hardness, X16 = springiness, X17 = cohesiveness, X18 = chewiness, X 19 = lipid oxidation, X20 = potassium, X21 = sodium, X22 = appearance, X23 = aroma, X24 = flavor, X25 = color, X26 = global impression.

for the sensory attributes of flavor and global impression, when compared to the treatments with higher levels of substitution.

4. Conclusions

The values for WLC, FE, and WE increased, but the values for hardness, springiness, cohesiveness and chewiness decreased, providing evidence of the fundamental action of sodium chloride in the solubilization of the myofibril proteins. All the samples were adequate for consumption concerning the bacteriological aspect, and the treatments with 15%, 30% and 45% of substitution of the NaCl by KCl were more accepted from a sensory point of view. The reduction of sodium by 1.05–9.2% using KCl to substitute NaCl, is possible in the spam-like products produced from MTM, allowing for the exploitation of this sub-product, but the product produced with 15% substitution of the NaCl by KCl can be considered to be the best product concerning the technological characteristics, presenting less fat and water exudation, less lipid oxidation and higher scores for the sensory attributes of flavor and global impression when compared with the treatments with greater levels of substitution of the NaCl by KCl.

CRediT authorship contribution statement

Elaine A. dos Santos: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing. **Alline Emannuele C. Ribeiro:** Methodology, Writing – review & editing. **Aryane R. Oliveira:** Methodology, Writing – review & editing. **Maria Lúcia G. Monteiro:** Writing – review & editing. **Eliane T. Mársico:** Conceptualization, Writing – review & editing, Supervision. **Marcelo Morgano:** Methodology, Writing – review & editing. **Márcio Caliari:** Conceptualization, Writing – review & editing, Supervision. **Manoel S. Soares Júnior:** Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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