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Textural, microstructural and sensory properties of reduced sodium frankfurter sausages containing mechanically deboned poultry meat and blends of chloride salts

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ABSTRACT

The purpose of this study was to investigate the effect of salt substitutes on the textural, microstructural and sensory properties in low-cost frankfurter sausages produced using mechanically deboned poultry meat (MDPM) as a potential strategy to reduce sodium in this meat product. In nine treatments, blends of calcium, sodium and potassium chloride were used to substitute 25% or 50% of the NaCl in sausages (at the same ionic strength and molar basis equivalent to 2% NaCl). When NaCl was partially replaced by 50% $CaCl_2$ (F6), the batter presented the highest total liquid released, and the corresponding final product showed increased hardness when compared to the control formulation (100% NaCl, C1). Its respective microstructural analysis revealed numerous pores arranged in a noncompacted matrix, which could be explained by the lowest emulsion stability reported for samples with 50% $CaCl_2$ and 50% NaCl. Frankfurter sausages produced using blends containing 25% $CaCl_2$ (F2, F4) and 25 and 50% KCl (F4, F5) did not exhibit differences in the pH, aw and objective color in the frankfurters ($p < 0.05$). The overall acceptability and flavor of the frankfurters containing 50% NaCl without salt substitution (C3), 25% $CaCl_2$, 25% KCl (F4), and 50% KCl (F5) showed the worst scores compared to those of the control formulation (C1). These results may be useful to select salt blends containing KCl, $CaCl_2$ and NaCl to reduce the sodium in sausages containing high concentrations of MPDM at an ionic equivalent strength to the NaCl commonly used in traditional formulations.

1. Introduction

Hypertension is a major risk factor for the development of various diseases, particularly cardiovascular diseases, and it represents a serious public health problem (WHO, 2007). Scientific studies have shown a correlation between these chronic diseases and excessive sodium intake, thus various government agencies have issued a recommended daily salt intake of only 5 g (2 g sodium) to reduce health risks (Weiss, Gibis, Schuh, & Salminen, 2010).

Recent studies have reported that meat products are responsible for approximately 20–30% of daily sodium consumption, which justifies the reduction of sodium chloride, a main ingredient used in processed meats, responsible for flavor, preservation, and textural properties (Petracci, Bianchi, Mudalal, & Cavani, 2013) being the major source of Na.

In meat emulsions, such as frankfurters, bologna, and pates, specific concentrations of sodium chloride are required to extract the myofibrillar proteins, which is soluble in high-ionic-strength solutions (greater than 0.3 M NaCl). The salted soluble meat proteins, are responsible for the water-holding capacity, emulsification and fat-binding properties in the batter and gel-forming stability during the cooking stage (Totosaus & Pérez-Chabela, 2009). In reduced-sodium emulsified meat products, the amount of myofibrillar protein extracted from the raw meat, therefore, will be a function of the salt content and type and the consequent level of dissociated ions (measured by the ionic strength) (Hultin, Feng, & Stanley, 1995).

Various strategies have been adopted by the industry to reduce the sodium content in meat products. KCl has been widely used to replace NaCl due to the ionic strength and chemical properties (Geleijnse, Kok, & Grobbee, 2003). However, KCl concentrations higher than 30–40% (depending on the formulation) result in a metallic taste, thus affecting the sensory properties of the product. The partial substitution of NaCl by $CaCl_2$ may, be a healthy alternative because it may provide additional calcium in the diet (Cáceres et al., 2006); however, many studies have reported that divalent salts such as $CaCl_2$, even with an NaCl equivalent ionic strength, can reduce the functional protein level in the batter, resulting in unstable emulsions (Ma et al., 2013; Piggot, Kenney, Slider, & Head, 2000).

Emulsified products, especially frankfurters, are widely consumed in Brazil. These products are characterized by the use of raw materials with high added value, such as mechanically deboned poultry meat (MDPM),

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whose addition is permitted up to 60%, according to the category of the product to be processed (MAPA, 2000); this makes it an excellent strategy for reducing cost.

The technological properties of MDPM in meat products depends on various factors, including the meat protein available, fat content, species and age of the animals, type of cut (neck, back, legs, wings), type of equipment, pretreatment of raw material (deboning, freezing), and operating conditions during the extraction process. In addition, the size and composition of the fat globules, pH, viscosity of the batter, and temperature increase during comminuting (Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2011) influence the quality of the meat emulsions, and these parameters are strongly affected by the MDPM content added to the formulations (Pereira et al., 2011).

Considering the importance of MDPM as a raw material in emulsified products and its technological properties, the aim of this study was to investigate the textural, microstructural and sensorial parameters of reduced-sodium frankfurter sausages produced with blends of sodium, potassium and calcium chloride and containing high levels of mechanically deboned poultry meat.

2. Material and methods

2.1. Treatments and formulations

Three control formulations with sodium reduction and without salt replacement were elaborated to evaluate the effect of salt substitutes: C1 (2% NaCl), C2 (1.5% NaCl) and C3 (1.0% NaCl). The others six treatments containing blends of NaCl, KCl and $CaCl_2$ substituting NaCl in 25–50%, are described in Table 1, according to the equivalent ionic strength. The following meat raw materials and additives were added to all formulations: mechanically deboned poultry meat (600 g/kg), pork meat (140 g/kg), pork back fat (100 g/kg), ice (100 g/kg), sodium nitrite (0.15 g/kg), sodium tripolyphosphate (3 g/kg), sodium erythorbate (4 g/kg), tapioca starch (20 g/kg), isolated soy protein (10 g/kg), and spices (4 g/kg). Three batches were prepared for each treatment.

2.2. Materials

Frankfurter sausages were prepared using lean pork shoulder meat (69.72% moisture, 10.66% fat and 18.28% protein), mechanically deboned poultry meat (MDPM — 67.89% moisture, 15.94% fat and 12.17% protein) and pork back fat (14.35% moisture, 76.28% fat and 8.47% protein) obtained from an industrial supplier (JBS, Brazil). The pork back fat and the pork meat were previously ground with a 5-mm disk. The spices, nonmeat ingredients and additives used in the sausages were obtained from various commercial and traditional suppliers (Fuchs, Brazil and New Max, Brazil). The salt blends added to the formulations were composed of food-grade NaCl, $CaCl_2$ and KCl (Merse, Brazil).

Table 1
Blends of salt substitutes (%) in frankfurters with strength ionic (IS) equivalent to NaCl 2%.

Treatment	NaCl		KCl		$CaCl_2$	
	IS	%	IS	%	IS	%
C1	0.342	2.00	0	0	0	0
C2	0.257	1.50	0	0	0	0
C3	0.171	1.00	0	0	0	0
F1	0.257	1.50	0.085	0.63	0	0
F2	0.257	1.50	0	0	0.085	0.31
F3	0.257	1.50	0.042	0.32	0.042	0.15
F4	0.171	1.00	0.085	0.63	0.085	0.31
F5	0.171	1.00	0.171	1.27	0	0
F6	0.171	1.00	0	0	0.171	0.63

2.3. Frankfurter-sausage processing

All frankfurter-sausage formulations were prepared in a pilot plant (UNICAMP, Brazil) according to the compositions of salts, raw materials and ingredients described in Sections 2.1 and 2.2. Both the pork meat and mechanically deboned poultry meat were placed into a cutter (Mado, model MTK 662, Germany), mixed with salt and half of the ice, and comminuted for 3–4 min at low speed for extraction of the myofibrillar proteins. When the temperature reached 7–8 °C, the other condiments and additives were slowly added so that the final temperature of the batter did not exceed 12 °C. The fat and the other half of the ice were added at the end of the process. Subsequently, the meat emulsion was embedded (Mainca, model EC12, Spain) in permeable cellulose wrappers (Viskase, ø 1 cm). The sausages were cooked in a steam oven (Arprotec, Brazil) with an initial temperature of 60 °C and relative humidity of 90–95% for 30 min. Then, the temperature was raised 5 °C every 10 min until reaching the final core of 72 °C. After cooking (~1 h and 15 min), the sausages were cooled in a cold-water shower and further in an ice bath. Then, the wrappers were removed, and the sausages were vacuum packed (Selovac, Minivac CU18) and stored under refrigeration (5 °C) before the physicochemical analyses.

2.4. Proximate analysis, sodium content, pH and aw

The moisture, protein, and ash contents were determined according to the Association of Official Analytical Chemists (AOAC, 2005). The fat content was determined by Soxhlet extraction with diethyl ether (AOAC, 2005). All tests were performed in triplicate. The mineral content (Na, K and Ca) was determined in triplicate for each formulation according to the methodology described by AOAC (2005). The pH determination was performed by homogenizing 10 g of each sample with distilled water in a 1:10 sample:water ratio in triplicate. The homogenate was subjected to a pH test using meter electrodes (DM 22, Digimed, São Paulo, Brazil) for 5 min while the pH readings were performed.

The water activity (aw) was measured by an Aqualab water activity meter (Decagon Devices Inc., Pullman, USA). Three frankfurters per treatment were used to evaluate aw, and each analysis was performed in triplicate at room temperature.

2.5. Emulsion stability

The emulsion stability tests of the various formulations were performed according to Jiménez Colmenero, Ayo, and Carballo (2005) with several modifications. Approximately 50 g of the batters formulated at a maximum temperature of 17 °C was weighed in centrifugation tubes and centrifuged (2600 rpm, 5 min at 5 °C). Subsequently, the samples were heated under two conditions: 40 °C for 15 min followed by 70 °C for 20 min. The tubes were left to stand upside-down for 45 min to release the exudates. The total amount of fluid released was expressed as a percentage of the sample weight. The content of released fat was determined by the difference in the total liquid released after drying in an oven at 100 °C for 16 h. In turn, the water released by evaporation was also expressed as a percentage of the sample weight. The test was performed in quintuplicate for each formulation.

2.6. Texture-profile analysis (TPA)

The texture-profile analysis of the sausages was performed at room temperature in quintuplicate using a texture analyzer (TA-xT2i Texture Technologies Corp., Scarsdale, NY), according to the methodology proposed by Bourne (2002). For calibration of the equipment, a load cell of 25 kg was used, and the test was performed by two successive compression cycles, considering 5 s as the time between compressions. Samples 2 cm thick and 1 cm in diameter were compressed to 30% of their original thickness, with a test speed of 1 mm/s, using a P-35 probe (35 mm in diameter, stainless steel). The parameters of hardness, cohesiveness, springiness and chewiness were evaluated. Hardness is the maximal force required to compress the sample, expressed in N. Cohesiveness is the ratio of the area during the second compression (A2) to that during the first compression (A1), dimensionless. The springiness ratio of the sample recovered after the first compression (dimensionless). The chewiness is expressed as the product of the gumminess and springiness, expressed in N.

2.7. Scanning electron microscopy

The microstructure of both the reduced-sodium sausages and the control sample was analyzed using scanning electron microscopy, according to the methodology proposed by Jiménez-Colmenero, Herrero, Pintado, Solas, and Ruiz-Capillas (2010), and Julavvittayanukul, Benjakul, and Visessanguan (2006),with modifications. The samples were cut into pieces of 3.2-mm thickness and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 4 h. The fixed samples were washed twice in phosphate buffer (0.1 M, pH 7.0) for 15 min. Subsequently, the samples were ground in liquid nitrogen and post-fixed in osmium tetroxide solution (1 g/100 g) for 2 h. The fixed samples were dehydrated in increasing concentrations of ethanol (30, 50, 70, 90 and 100% for 30 min). Then, the samples were critically-point dried and mounted onto a stainless steel sample holder, coated with gold and observed using a JEOL JSM 5800LV scanning electron microscope (JEOL Ltd., Tokyo, Japan) at a 20-kV accelerating voltage.

2.8. Sensory analysis

The frankfurter samples were evaluated by 100 untrained assessors, comprising students and staff of the University of Campinas. The sensory analysis protocol for the frankfurters was previously approved by the Ethics in Research Committee of the State University of Campinas, SP, Brazil, under protocol number 206.073/2012. The acceptance attributes were appearance, aroma, flavor, texture and overall impression. The samples were served serially, and the order of presentation followed a completely balanced block (Macfie, Bratchell, Greenhoff, & Vallis, 1989). Sausages were immersed in boiling water and kept at this temperature for 3 min, and then served in the temperature range of 35–40 °C. The acceptance values were measured on an unstructured nine-point hedonic scale (Meilgaard, Civille, & Carr, 1999).

2.9. Statistical analysis

For each treatment, three batches were used, and the data were analyzed by using SPSS (v. 19, IBM SPSS Inc., Chicago, IL) for two-way ANOVA to determine differences ($p < 0.05$) with Tukey's HSD test. For the sensory study, the results were analyzed using the SAS statistical software (SAS Institute, 2003) by ANOVA and Tukey's test (one-way) at a 5% significance level.

3. Results and discussion

3.1. Composition, pH, aw, emulsion stability

Reduced-sodium frankfurters formulated with blends of KCl, $CaCl_2$ and NaCl were characterized for their physicochemical properties. The control formulation C1 had the following chemical composition: moisture (59.85%), fat (21.49%), protein (12.03%), and ash (3.60%) contents. All other formulations were prepared using raw materials and ingredients from the same batch, obtained from the same supplier, varying only the content of the salt substitutes.

The pH of the batters and frankfurters, as well as the values of the water activity (aw), emulsion stability (total liquid, fat, and water released) and sodium contents are shown in Table 2. For all treatments (C1–F6), the batter pH values varied from 6.02 (F6) to 6.36 (C3). The addition of $CaCl_2$ to the blends of the salts resulted in a decrease in the pH of both the batter and the final product ($p < 0.05$). Similar results were found by Gimeno, Astiasaran, and Bello (1999). Piggot et al. (2000) reported that the pH of beef emulsions with dicationic salts added decreased in raw sausages, but the cooked sausage pH was unaffected. According to Sun and Holley (2010), at the isoelectric point (Ip) of the myofibrillar protein (pH 5.3), either only poor gels are formed or gel formation is inhibited. At pH 6, the optimum pH value for heat-induced gelation of myosin is reached. Then, despite this decrease in the pH observed for treatments containing $CaCl_2$, the values reported in this study are far from the isoelectric point of myosin (5.3), and this parameter alone is not enough to exclude $CaCl_2$ from the blends used to reduce NaCl in emulsified meat products.

The emulsion stability for various batters of reduced-sodium frankfurters was analyzed by % total liquid and fat released. The most stable batters concerning the total liquid released were the control formulation C1 and C2 and the formulations containing blends of NaCl and KCl, which are composed of monovalent ions (F1 and F5). It was observed that simply reducing the value to 50% NaCl (C3) resulted in a significant decrease in the emulsion stability ($p < 0.05$). The treatment F6 followed by the sample F3 showed higher values of released fat in the emulsion-stability test when compared to the control sample (C1), showing a low fat-binding capacity for the protein.

Many studies have reported that the presence of divalent calcium ions can contribute to reduced myofibrillar protein extraction, responsible for the fat and water binding in the meat batter when compared to monovalent chlorides. The inability of $CaCl_2$, to extract substantial amounts of myofibrillar proteins from meat may be due to its charge density or specific ion effects (Gordon & Barbut, 1992). According to Horita, Morgano, Celeghini, and Pollonio (2011), the addition of $CaCl_2$ may reduce the emulsion stability and cooking yield in the emulsified

Table 2
pH of the batter and final product, water activity, emulsion stability of reduced sodium frankfurters added of blends of salts substitutes.

Treatment	Raw sausage pH	Cooked sausage pH	aw	Emulsion stability		
				% total liquid released	% water released	% fat released
C1	6.29^{c} (0.03)	6.34^{b} (0.03)	0.97^{a} (0.00)	1.95^{e} (0.33)	1.71^{e} (0.29)	0.23^{f} (0.05)
C2	6.28^{d} (0.01)	6.28^{d} (0.01)	0.97^{a} (0.00)	2.38^{e} (0.50)	2.12^{e} (0.45)	$0.27^{e,f}$ (0.05)
C3	6.36^{a} (0.03)	6.39^{a} (0.04)	0.97^{a} (0.01)	8.52^{b} (1.09)	7.94^{b} (0.99)	0.58^{b} (0.18)
F1	6.32^{b} (0.02)	6.32^{c} (0.02)	0.97^{a} (0.00)	2.82^{e} (0.46)	2.46^{e} (0.39)	$0.36^{c,d,e}$ (0.07)
F2	6.12^{f} (0.01)	6.12^{f} (0.01)	0.97^{a} (0.00)	$5.26^{c,d}$ (1.80)	$4.81^{c,d}$ (1.63)	0.45^{c} (0.18)
F3	6.19^{e} (0.01)	6.21^{e} (0.03)	0.97^{a} (0.01)	4.26^{d} (1.97)	3.87^{d} (1.80)	$0.39^{c,d}$ (0.18)
F4	6.14^{f} (0.01)	6.13^{f} (0.01)	0.97^{a} (0.00)	5.74^{c} (0.53)	5.13^{c} (0.47)	0.61^{b} (0.06)
F5	6.33^{b} (0.01)	$6.33^{b,c}$ (0.01)	0.97^{a} (0.00)	2.54^{e} (0.40)	2.24^{e} (0.35)	$0.29^{d,e,f}$ (0.05)
F6	6.02^{g} (0.02)	6.01^{g} (0.02)	0.97^{a} (0.00)	16.88^{a} (0.70)	15.27^{a} (0.63)	1.61^{a} (0.08)

Values are means (standard deviation).
Same letters within the same column do not differ significantly ($p < 0.05$) according to Tukey's test.
C1: 100% NaCl; C2: 75% NaCl; C3: 50% NaCl; F1: 75% NaCl + 25% KCl; F2: 75% NaCl + 25% $CaCl_2$; F3: 75% NaCl + 12.5% $CaCl_2$ + 12.5% KCl; F4: 50% NaCl + 25% $CaCl_2$ + 25% KCl; F5: 50% NaCl + 50% KCl; F6: 50% NaCl + 50% $CaCl_2$.

meat products, and so it is necessary to use various nonmeat ingredients as thickeners to improve these properties. As previously observed, the increased pH in batters containing $CaCl_2$ probably was not the main factor for the lower emulsion stability of the treatment F6 (50% $CaCl_2$) which showed the highest level of liquid released (16.88 %) when compared to the other formulations. Comfort and Howell (2003) studied the gelation properties of salt-soluble meat protein and soluble wheat protein mixtures, and observed that, with the addition of sodium, potassium, and calcium chloride, the gel strength of salt-soluble beef protein was the strongest in the presence of potassium, followed by sodium, and then calcium chloride. No differences were observed for the water-activity values.

3.2. Sodium, potassium and calcium contents

The contents of Na, Ca and K shown in Table 3 are in accordance with the expected results. A 50% reduction in NaCl resulted in an approximately 35% sodium reduction. It should be noted that other components contribute to the sodium in the formulations, such as sodium tripolyphosphate, sodium nitrite, sodium erythorbate, and the meat raw materials. This makes it even more difficult to reduce sodium by simply decreasing the sodium chloride content because for consistent reformulation, the NaCl content must be drastically reduced, thus influencing the sensory and technological properties and the food safety.

Regarding the calcium contents, all treatments containing blends without calcium chloride shown amounts of approximately 255–270 mg/100 g sample due to the use of MDPM, which is characterized by high levels of residual bone fragments resulting from the deboning process. Formulations without the addition of blends containing KCl (C1, C2, C3, F2 and F6) showed lower values for the potassium content (approximately 230 mg/100 g product), but with significant differences ($p < 0.05$). These significant differences ($p < 0.05$) can be explained by the heterogeneity of the meat batters that are not a true emulsion. As can be observed from the results for Na, Ca and K, the use of blends of chloride salts as partial substitutes for NaCl could favor a balanced intake of minerals from sausage, which is quite evident in the formulations F3 and F4.

3.3. Texture-profile analysis

In emulsified meat products such as frankfurters and bologna sausages, the texture is one of the most important sensory attributes and is directly related to the fat and water-holding capacities of the meat matrix, which in turn, are influenced by the ionic strength and functional properties of the proteins (Hamm, 1986). Thus, when the NaCl level is reduced, the amount of soluble myofibrillar proteins can also decrease (Gordon & Barbut, 1992) as consequence of the reduced ionic strength or by changing in charge density or specific ion effects from others salts, thereby decreasing the water-holding capacity and gel strength (Whiting, 1984).

Table 4 shows the texture-profile analysis and, as expected, both formulations C1 and C2 had the lowest hardness values. Moreover, it was observed that all formulations containing 25% or 50% $CaCl_2$ as salt substitute showed the highest hardness values and were different from the control formulation with 100% NaCl ($p < 0.05$). The highest value for this parameter was assigned to the formulation F6 containing 50% $CaCl_2$, which also had the highest levels of liquid released in emulsion stability test, confirming the negative contribution of the divalent salt $CaCl_2$ to texture properties. In emulsified meat products, the increase in the hardness is attributed to the reduction of bound water in the batter during cooking. Similar results were reported by Totosaus and Pérez-Chabela (2009) and Horita et al. (2011). According to those authors, this is due to the low gel compression observed in the protein matrix. In this study, the use of high MDPM levels makes more obvious this phenomenon. It is also observed that the formulations containing 25% and 12.5% $CaCl_2$ (F2, F3, F4) were not different from the formulation C3, with 50% NaCl replacement, which was considered the most critical formulation due to its lower ionic strength ($p < 0.05$).

Replacement of 50% of NaCl by KCl (F5) resulted in higher hardness values than those found in the control formulation (C1 − 100% NaCl). However, the formulation F1, in which only 25% NaCl was replaced by KCl, did not present different hardness values from the control formulation C1. When working with commercial formulations that have high MDPM levels, it is desirable to optimize the salt content to ensure sensory acceptance when using KCl, even at low levels. The mechanically deboned meat provides a residual metallic taste due to its high iron content from the mechanical deboning process. Studies suggest that, although $CaCl_2$ has good sensory properties, its application must be optimized to ensure the physical and microbiological stability (Seman, Olson, & Mandigo, 1980).

Because chewiness is markedly influenced by hardness, the interpretation of the results can be similar. For the attribute elasticity, no differences were reported for all formulations ($p < 0.05$). With respect to the cohesiveness, the samples containing blends showed higher values ($p < 0.05$) than the control sample C1.

3.4. Objective color determination

The values of L* (luminosity), a* (red-color intensity) and b* (yellow-color intensity) are reported in Table 5. The reduction of sodium chloride provided significant differences in the L* values, tending to increase the

Table 3
Sodium, potassium and calcium contents of reduced sodium frankfurters added of blends of salt substitutes.

Treatment	Element (mg/100 g)		
	Sodium	Potassium	Calcium
C1	1113.75 (35.61)^{a}	223.17 (4.73)^{e,f}	259.12 (9.72)^{c,d}
C2	794.58 (12.90)^{b}	204.42 (2.89)^{f}	200.08 (2.64)^{f}
C3	673.11 (7.11)^{c}	200.06 (1.85)^{f}	276.10 (5.67)^{c}
F1	849.02 (4.51)^{b}	601.35 (3.18)^{b}	274.94 (4.37)^{c}
F2	852.03 (9.00)^{b}	261.84 (1.58)^{d,e}	312.36 (2.86)^{b}
F3	815.86 (0.73)^{b}	357.17 (12.74)^{c}	240.39 (11.32)^{d,e}
F4	674.44 (16.56)^{c}	594.72 (13.21)^{b}	291.78 (10.43)^{b,c}
F5	672.20 (22.85)^{c}	882.87 (41.26)^{a}	225.66 (13.57)^{e,f}
F6	671.33 (10.80)^{c}	282.32 (7.88)^{d}	411.56 (4.10)^{a}

Values are means (standard deviation).
Same letters within the same column do not differ significantly ($p < 0.05$) according to Tukey's test.
C1: 100% NaCl; C2: 75% NaCl; C3: 50% NaCl; F1: 75% NaCl + 25% KCl; F2: 75% NaCl + 25% $CaCl_2$; F3: 75% NaCl + 12.5% $CaCl_2$ + 12.5% KCl; F4: 50% NaCl + 25% $CaCl_2$ + 25% KCl; F5: 50% NaCl + 50% KCl; F6: 50% NaCl + 50% $CaCl_2$.

Table 4
Texture profile analysis (TPA) of reduced sodium frankfurters added of blends of salt substitutes.

Treatment	Hardness (N)	Springiness (dimensionless)	Cohesiveness (dimensionless)	Chewiness (N)
C1	10.57^{e} (1.27)	0.91^{a} (0.02)	0.80^{c} (0.02)	7.75^{c} (0.88)
C2	11.07^{d,e} (1.07)	0.91^{a} (0.02)	0.83^{a} (0.01)	8.40^{b,c} (1.49)
C3	12.43^{b,c} (1.27)	0.91^{a} (0.03)	0.81^{b,c} (0.03)	7.75^{b,c} (1.70)
F1	10.40^{e} (0.79)	0.91^{a} (0.03)	0.82^{a,b,c} (0.03)	7.75^{b,c} (0.70)
F2	13.37^{a,b} (1.07)	0.91^{a} (0.02)	0.81^{a,b,c} (0.02)	9.95^{a} (0.76)
F3	12.47^{b,c} (1.48)	0.91^{a} (0.02)	0.82^{a,b,c} (0.02)	9.23^{a} (1.17)
F4	12.28^{b,c,d} (1.11)	0.91^{a} (0.02)	0.83^{a,b} (0.02)	9.30^{a} (0.87)
F5	12.07^{c,d} (1.28)	0.91^{a} (0.03)	0.83^{a,b} (0.03)	9.21^{a} (1.10)
F6	14.04^{a} (1.27)	0.89^{a} (0.02)	0.82^{a,b,c} (0.02)	9.37^{a} (0.95)

Values are means (standard deviation).
Same letters within the same column do not differ significantly ($p < 0.05$) according to Tukey's test.
C1: 100% NaCl; C2: 75% NaCl; C3: 50% NaCl; F1: 75% NaCl + 25% KCl; F2: 75% NaCl + 25% $CaCl_2$; F3: 75% NaCl + 12.5% $CaCl_2$ + 12.5% KCl; F4: 50% NaCl + 25% $CaCl_2$ + 25% KCl; F5: 50% NaCl + 50% KCl; F6: 50% NaCl + 50% $CaCl_2$.

Table 5
L*, a* and b* values of reduced sodium frankfurters added of blends of salt substitutes.

Treatment	L*	a*	b*
C1	60.03^{d} (1.05)	14.03^{a} (0.70)	$15.30^{a,b}$ (0.49)
C2	$62.04^{a,b,c}$ (1.00)	14.06^{a} (0.51)	15.62^{a} (0.23)
C3	61.13^{c} (0.73)	$13.80^{a,b}$ (0.29)	15.21^{b} (0.33)
F1	60.03^{d} (0.77)	14.18^{a} (0.46)	$15.37^{a,b}$ (0.24)
F2	$62.19^{a,b}$ (0.61)	$13.72^{a,b}$ (0.38)	$15.36^{a,b}$ (0.22)
F3	62.92^{a} (0.62)	13.30^{b} (0.43)	$15.36^{a,b}$ (0.36)
F4	$62.12^{a,b}$ (0.92)	14.00^{a} (0.45)	$15.48^{a,b}$ (0.28)
F5	$61.47^{b,c}$ (0.92)	$13.87^{a,b}$ (0.54)	$15.53^{a,b}$ (0.23)
F6	$61.41^{b,c}$ (0.38)	14.15^{a} (0.50)	$15.28^{a,b}$ (0.210)

Values are means (standard deviation).
Same letters within the same column do not differ significantly ($p < 0.05$) according to Tukey's test.
C1: 100% NaCl; C2: 75% NaCl; C3: 50% NaCl; F1: 75% NaCl + 25% KCl; F2: 75% NaCl + 25% $CaCl_2$; F3: 75% NaCl + 12.5% $CaCl_2$ + 12.5% KCl; F4: 50% NaCl + 25% $CaCl_2$ + 25% KCl; F5: 50% NaCl + 50% KCl; F6: 50% NaCl + 50% $CaCl_2$.

brightness of the formulations containing $CaCl_2$ (F2, F3, F4), without significant differences ($p < 0.05$). Similar findings have been presented in work of Boyle, Addis, and Epley (1994), who studied sausages fortified with calcium, and Gimeno et al. (1999), who investigated a fermented meat product with sodium reduction using a 2.29% salt blend (1.00% NaCl, 0.55% KCl, and 0.74% $CaCl_2$), which showed higher L* values compared to those of the control formulation. In addition, they concluded that the L* values increased when calcium was incorporated into the formulation. Arganosa and Marriott (1990) found lighter luncheon meat when compared to the control after the addition of magnesium and calcium chloride for a 50% ionic strength replacement. The authors concluded that calcium chloride was detrimental to this quality attribute.

The salt substitutes did not affect the parameter a* (red-color intensity), with no differences between the formulations studied. The same was observed for the parameter b* (yellow-color intensity). It is likely that the protein–water interactions discussed above may have contributed to the color differences of the sausages.

3.5. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was conducted in this study to investigate the interfacial protein film morphology and protein matrix structure. In this study, the main goal is correlating the SEM data with textural properties to describe the influence of salt substitutes to NaCl on the cooked batters.

The microstructures are presented in Fig. 1. Image C, which corresponds to the formulation C3 containing the lowest salt content (50% NaCl) showed an open and spongy (a large number of pores) structure, in part due to the released water from the low salt meat matrix when compared to that of the control formulation (Image A) with the highest salt content (C1).

The highest extraction of the myofibrillar proteins in the formulation containing higher sodium chloride content, and ionic strength resulted in a dense protein matrix that was porous homogeneous and had excellent cohesiveness between the continuous and dispersed phases. Therefore, when the sodium chloride content was reduced and not replaced by others salts, the amount of extracted muscle protein decreased, consequently lowering the water-binding ability and gel strength (Gordon, 1993). In fact, reducing the NaCl content from 2% to 1% increased both the losses during cooking and the hardness of the product, which was verified by the texture-profile analysis.

Image B representing the formulation C2 revealed compact, nonporous protein matrix with dense characteristics similar to those of the

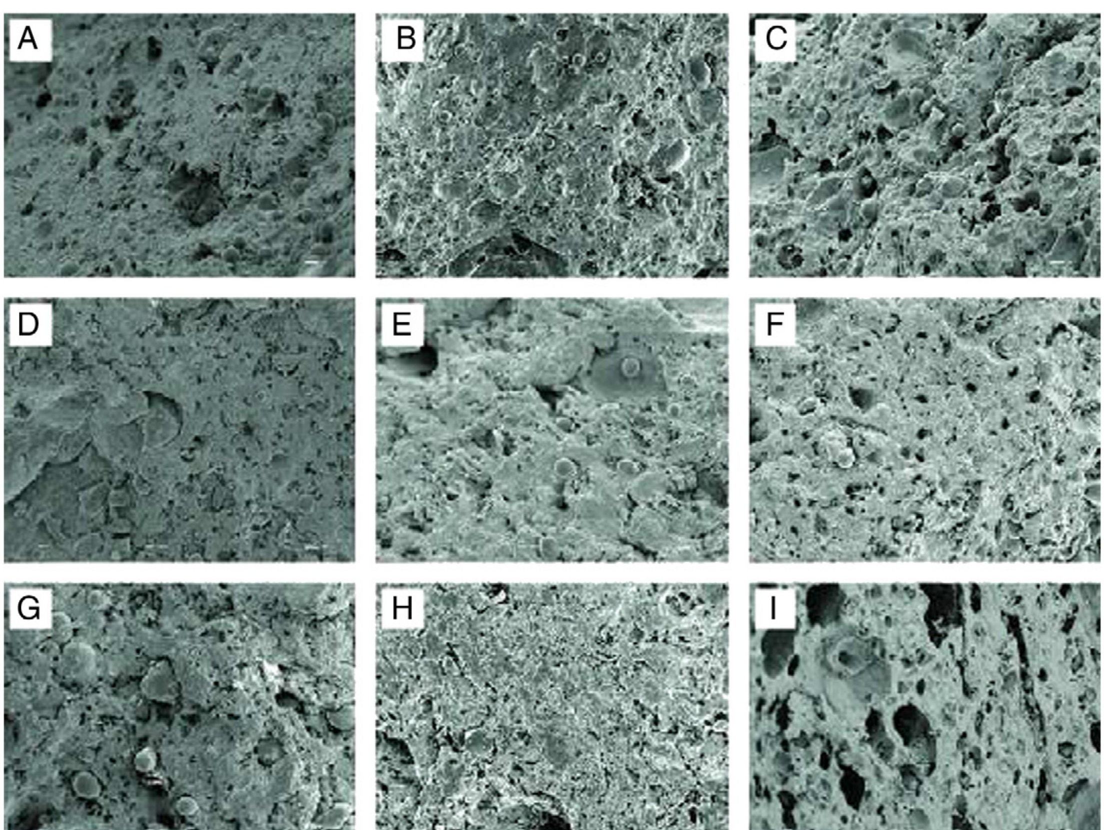

Fig. 1. Scanning electronic microscopy of the frankfurters. Bar represents 10 μm. A) C1: 100% NaCl; B) C2: 75% NaCl; C) C3: 50% NaCl; D) F1: 75% NaCl + 25% KCl; E) F2: 75% NaCl + 25% $CaCl_2$; F) F3: 75% NaCl + 12.5% $CaCl_2$ + 12.5% KCl; G) F4: 50% NaCl + 25% $CaCl_2$ + 25% KCl; H) F5: 50% NaCl + 50% KCl; I) F6: 50% NaCl + 50% $CaCl_2$.

control C1. Similar results can be seen in Fig. 1D (F1) and Fig. 1H for (F5) containing only KCl as a salt substitute with an equal ionic strength which, in turn, showed a higher emulsion stability among the treatments.

From the functional point of view, it was evident that $CaCl_2$ negatively contributed to the blend, causing emulsion instability and poor water retention. Microstructure I which had a higher $CaCl_2$ content in the blend (50%), resulted from the formulation that presented the lowest emulsion stability, as evidenced by the numerous pores (irregular and with varied sizes), probably due to the release of fat and water. However, images E and G and F containing lower $CaCl_2$ contents (25 and 12.5%, respectively), presented a structure more homogeneous with a lower pore content and size.

Several hypotheses are reported to justify the lower stability of the batter formulated with blends containing $CaCl_2$ and smaller water-holding capacities, such as (1) the calcium chloride content, which extracted significantly less of the total protein from the meat (Gordon & Barbut, 1992); (2) the greater volume of the cation Ca^{2+} (Tang, Tung, & Zeng, 1997); (3) the decreased gel strength (Totosaus & Pérez-Chabela, 2009); (4) the greater input of chloride ions in the meat matrix (Hamm, 1986); (5) the weakening of the bonds between the molecules (protein–water, protein–fat) (Whiting, 1984); (6) the reaction of chloride with calcium phosphate (Irani & Callis, 1962); and (7) a reduction in the pH of the emulsion, thus causing it to approach the isoelectric point of myosin and decreasing its functionality (Claus, Hunt, & Kastner, 1989).

From the technological point of view, F1 and C1 were the most stable formulations. These results could be relevant to elaborate recommendations regarding the composition of blends of salt substitutes to reduce sodium in frankfurters containing high levels of MDPM. However, to support this conclusion, comparative studies of the sensory and microbiological stability of the product during the shelf life are necessary.

3.6. Sensory analysis

The sensorial acceptance test was conducted by 100 untrained consumers, including undergraduate students, postgraduate students and employees of the State University of Campinas, representing a target public that consumes frankfurter sausages at least once per week. Table 6 shows the values for each of the analyzed attributes: appearance, aroma, flavor, texture and overall acceptance.

It was generally observed that formulation C1 presented the highest value for all attributes, demonstrating that the consumers, despite not being trained, were able to identify the control treatment among the samples containing various blends of salt substitutes to NaCl.

Regarding appearance and aroma, there was no difference ($p < 0.05$) for all formulations and controls when compared to C1. For texture, the lowest score among the control formulations and samples with blends was shown by C3, which had no substitute salt added. This is due to a major reduction in the salt content and ionic strength, which resulted in a lower water-holding capacity and a weak, porous and fragile meat batter (Aaslyng, Vestergaard, & Koch, 2014).

Concerning flavor, one of the most important attributes to select the best blend of salt substitutes for NaCl, C2 and C3, without KCl or $CaCl_2$ added and with 25 and 50% NaCl reduction, presented the lowest scores. This is in agreement with the salted and enhanced global flavor resulting from the diminished NaCl concentration (Tobin, O'Sullivan, Hamill, & Kerry, 2012).

The effect of KCl as a blend component can be explained by the lowest score for F5, containing 50% KCl added as the salt substituted. Many authors have reported that KCl over 30% of substitution results in a bitter and metallic taste (Aaslyng et al., 2014; Petracci et al., 2013). C3 was not different from F5, indicating the negative effect of a high substitution level of KCl. However, when only 25% NaCl substitution or reduction is performed in blends, the frankfurters are not affected in flavor, even using KCl as a salt substitute.

The use of 25 (F2 and F4) or 50% $CaCl_2$ (F6) to substitute NaCl revealed a potential sensorial solution to reduce Na in the low-cost frankfurter sausage since such formulations were not different from control (C1) in flavor ($p < 0.05$) However, $CaCl_2$ results in a low emulsion stability when used for 50% NaCl substitution, indicating that other ingredients to improve the water-holding capacity, such as extenders, could be added to compensate for these negative effects on the reduced-NaCl batter. The overall acceptance followed the same tendencies reported for the other sensorial attributes: the control formulation was the most accepted, and the samples containing 50% KCl (F5) were the worst.

Similar findings have been presented in other work (Choi et al., 2014; Horita et al., 2011).

4. Conclusion

The use of various blends of salt substitutes containing KCl, $CaCl_2$ and NaCl at ionic strengths equivalent to that of the salt commonly used in traditional formulations appears to be a good and reliable strategy to reduce the sodium in sausages containing high concentrations of mechanically deboned poultry meat. The presence of $CaCl_2$ as a partial salt substitute in the blends negatively contributed to the functional properties of the products because it provided lower emulsion stability, as evidenced by the numerous porous in the microstructure, confirming the water release and the lower stability of the batter. From a technological point of view, KCl is the best salt substitute in sausages containing high concentrations of mechanically deboned poultry meat. In addition, formulations with 25% NaCl replaced by salt blends of $CaCl_2$ and KCl presented sensory performance similar to that of the control sample.

Table 6
Sensory study of frankfurters of reduced sodium frankfurters added of blends of salt substitutes.

Treatments	Appearance	Aroma	Taste	Texture	Overall impression
C1	5.17 (2.12)^{a}	5.64 (2.08)^{a}	6.19 (1.82)^{a}	5.95 (2.18)^{a}	5.81 (1.92)^{a}
C2	4.84 (2.20)^{a}	5.24 (1.97)^{a}	5.24 (1.90)^{b,c,d}	5.41 (1.90)^{a,b}	5.19 (1.72)^{a,b,c}
C3	4.46 (2.08)^{a}	4.99 (1.99)^{a}	4.48 (2.05)^{d}	4.86 (2.17)^{b}	4.59 (1.74)^{c}
F1	5.09 (2.21)^{a}	5.63 (2.05)^{a}	5.61 (2.00)^{a,b,c}	5.54 (2.03)^{a,b}	5.43 (1.78)^{a,b}
F2	5.24 (1.98)^{a}	5.37 (1.95)^{a}	6.05 (1.66)^{a,b}	5.22 (2.05)^{a,b}	5.60 (1.55)^{a,b}
F3	4.96 (1.89)^{a}	5.66 (1.96)^{a}	5.74 (1.90)^{a,b,c}	5.34 (2.22)^{a,b}	5.45 (1.70)^{a,b}
F4	5.10 (2.18)^{a}	5.63 (2.01)^{a}	5.29 (2.02)^{b,c,d}	5.73 (2.15)^{a,b}	5.28 (1.88)^{a,b,c}
F5	4.76 (1.89)^{a}	5.05 (2.10)^{a}	5.12 (1.93)^{c,d}	5.08 (2.23)^{a,b}	4.92 (1.71)^{b,c}
F6	4.89 (2.24)^{a}	5.28 (2.06)^{a}	5.40 (2.11)^{a,b,c}	5.20 (2.06)^{a,b}	5.13 (1.79)^{a,b,c}

Values are means (standard deviation).
Same letters within the same column do not differ significantly ($p < 0.05$) according to Tukey's test.
C1: 100% NaCl; C2: 75% NaCl; C3: 50% NaCl; F1: 75% NaCl + 25% KCl; F2: 75% NaCl + 25% $CaCl_2$; F3: 75% NaCl + 12.5% $CaCl_2$ + 12.5% KCl; F4: 50% NaCl + 25% $CaCl_2$ + 25% KCl; F5: 50% NaCl + 50% KCl; F6: 50% NaCl + 50% $CaCl_2$.

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