PAPER

FUNCTIONAL SAUSAGE MADE FROM MECHANICALLY SEPARATED TILAPIA MEAT

D. PEIREIRA BESSA¹, C.E. TEIXEIRA¹, R. MAIA FRANCO¹, M. QUEIROZ DE FREITAS¹, M.L. GUERRA MONTEIRO^{1*}, C.A. CONTE-JUNIOR¹, L. VARON GAZE¹, F. ALVES DA SILVA² and E. TEIXEIRA MÁRSICO¹

¹Departament of Food Technology, Federal Fluminense University, Niterói, Rio de Janeiro, Brazil, 24230-340 ²Departament of Food Engineering, Federal de Goiás University, Rod. Goiânia/Nova Veneza, Km 0, Goiânia, GO, Brazil, 74690-900

*Corresponding author: Tel.: + 55 02126299545; fax: + 55 02126299541 E-mail address: marialuciaguerra@yahoo.com.br

ABSTRACT

Physicochemical, bacteriological and sensory parameters of sausages made from waste of Nile tilapia with the prebiotic inulin added and reduced sodium were investigated. Mechanically separated carcass meat and mechanically separated head meat were processed into sausages with or without inulin and salt replacer. T1 and T3 showed greatest lipid, but lowest carbohydrate levels, a^* and b^* values (P < 0.05). In general, the inulin formulations showed higher acceptability and purchase intent (P < 0.05). The addition of inulin to low-sodium tilapia sausages is a promising technological strategy to minimize negative effects on the taste and texture from KCl increment.

Keywords: fish waste, healthy products, inulin, *Oreochromis niloticus*, potassium chloride, salt replacer

1. INTRODUCTION

The Nile tilapia is the most important species for aquaculture in the world. Tilapia is marketed either as whole fish or as fresh or frozen fillets, although consumers prefer fillets. Reported fillet yields of Nile tilapia vary widely (30-40%), generating a large amount of wastes that are commonly underutilized, used as animal feed, or discarded (MONTEIRO *et al.*, 2012; MONTANHINI NETO and OSTRENSKY, 2013).

The utilization of fish-processing waste to develop new products provides an important opportunity for the food industry to produce sustainable and value-added products as a source of omega-3 polyunsaturated fatty acids (DECKELBAUM and TORREJON, 2012) and high-value protein. Further studies are needed to develop ways to use fish waste to produce functional products with lower costs (MONTEIRO *et al.*, 2014a; PALMEIRA *et al.*, 2014b).

Some investigators have reported the beneficial effects of including inulin as a fat replacer in chicken and pork sausages, with desirable effects such as improved texture. In addition, inulin has a prebiotic physiological effect, attributed to fructan, which stimulates the growth of beneficial bacteria in the intestine of the host (MENDOZA *et al.*, 2001; MENEGAS *et al.*, 2013).

The reduction of sodium in processed products has constituted a challenge for the food industry, particularly in view of the contemporary consumer's awareness of the relationship between food and health. The need to reduce salt contents is further reinforced by agreements between the industrial sector and the World Health Organization (WHO). The main challenge is to maintain the acceptability as well as physicochemical parameters of the food (DESMOND, 2006; DOTSCH *et al.*, 2009; MONTEIRO *et al.*, 2014b), considering microbiological quality standards. However, inulin and sodium replacers in fish products have not been studied.

This study evaluated the effect of sodium reduction and inulin addition on physicochemical, bacteriological and sensory parameters of sausages manufactured from mechanically separated meat of Nile tilapia.

2. MATERIALS AND METHODS

2.1. Obtaining mechanically separated tilapia meat

The wastes from heads and carcasses of tilapia were obtained from a fish-farming cooperative in Rio de Janeiro, Brazil. The fish heads and carcasses were passed through a deboning machine (KME, São Paulo) to remove the muscle from the bones, and were then washed.

The mechanically separated carcass meat (MSCM) and mechanically separated head meat (MSHM) were frozen and packed in 1kg polyethylene packages at -18°C. The samples were transported in cold boxes to keep them frozen during transport to the pilot plant of the Centro de Tecnologia SENAI Alimentos e Bebidas, Rio de Janeiro, Brazil, where they were stored in a freezer (-18°C) until the MSHM and MSCM were used to produce sausages. Four different sausage formulations were prepared: 100% NaCl without inulin (T1), 100% NaCl + 6% inulin (T2), 50% NaCl and 50% KCl + without inulin (T3), and 50% NaCl and 50% KCl + 6% inulin (T4). In formulations without inulin, MSHM was used as a replacer, and potassium chloride (KCl) was used to reduce the sodium content. All formulations are described in Table 1.

la una dia sata (0()		Treat	ments*	
ingredients (%)	T1	T2	Т3	Τ4
MSCM**	81.91	81.91	81.91	81.91
Sodium chloride	1.3	1.3	0.65	0.65
Potassium chloride	-	-	0.65	0.65
Soybean protein	2.0	2.0	2.0	2.0
MSHM**	10.0	4.0	10.0	4.0
Inulin	-	6.0	-	6.0
Polyphosphate	0.5	0.5	0.5	0.5
Manioc starch	2.0	2.0	2.0	2.0
Sodium erythorbate	0.3	0.3	0.3	0.3
Carmine	0.05	0.05	0.05	0.05
Collagen	1.0	1.0	1.0	1.0
Nitrite	0.25	0.25	0.25	0.25
Onion	0.3	0.3	0.3	0.3
Garlic	0.2	0.2	0.2	0.2
White pepper	0.1	0.1	0.1	0.1
Cilantro	0.05	0.05	0.05	0.05
Ginger	0.04	0.04	0.04	0.04

Table 1: Formulations of sausages manufactured from mechanically separated tilapia meat.

*T1 (100% NaCl without inulin), T2 (100% NaCl + 6% inulin), T3 (50% NaCl and 50% KCl + without inulin), T4 (50% NaCl and 50% KCl + 6% inulin).

** MSCM - mechanically separated carcass meat; and MSHM - mechanically separated head meat.

2.2. Experimental design

Ten kg and thirty-kg of MSHM and MSCM were obtained, respectively. For each experimental replication (n = 2), the samples were divided into four groups each with 3.5 kg of MSHM (T1 and T3), 1.5 kg of MSHM (T2 and T4), and 7.5 kg of MSCM. Each group was divided at random into 40 g portions, resulting a total of 40 samples per group which were separated in the proposed formulations and analyzed on days 0 and 34 of refrigerated storage, totaling 80 samples units.

2.3. Sausage manufacturing

To compose the sausage, the ingredients (Table 1) were mixed in a cutter. After the emulsion was formed in the cutter, the sausage mass was placed in a manual sausage maker using sausage collagen casings of 21 mm diameter, and separated into sections. The sausage was heat-treated, starting with pre-cooking in a drying oven (Incomaf Indústria Ltda., São Paulo, Brazil) with circulating hot air at 50°C for 15 min with an open chimney, then raised to 60°C for 30 min with a closed chimney and increased by 5°C every 5 min (steam drying) until the sausage reached 72°C internally, as measured with the aid of a thermocouple. The sausages were then cooled in a water bath to an internal temperature

of 40°C. The sausages were stored in a cooling chamber at 5°C until the next day, when the membranes were delaminated and the products were vacuum-packed in polythene bags and stored under refrigeration (5°C). The products were placed in isothermal boxes and transported to the laboratory in Rio de Janeiro, Brazil, where they were kept under refrigeration (4°C) for 34 days until analytical procedures. The analyses on day 0 were performed immediately after the arrival of the samples.

2.4. Physicochemical analyses

Water, ash, protein and lipid contents were evaluated in MSHM and MSCM on day 0 (AOAC, 2012). In the sausages, pH values were determined with a digital pH meter (Digimed®DM-22) equipped with a DME-R12 electrode (Digimed®) according to CONTE-JÚNIOR *et al.* (2008). Water, ash, protein, lipid and carbohydrate contents were determined by standard methods (AOAC, 2012). The carbohydrate content was estimated taking into account the ingredient composition. The energy content (kcal/100g) was estimated according to TRIKI *et al.* (2013). These analyses were performed immediately after manufacture and repeated after 34 days of storage, to assess the maintenance of quality and identity of the product.

In addition, on day 0 the cooking yield was calculated as the cooked weight of the sausages divided by the weight of the pre-cooked sausage, multiplied by 100 (HORITA *et al.*, 2011). All analyses were performed in quadruplicate for each treatment.

The instrumental color parameters were determined using a Konica Minolta CR-400 colorimeter (Konica Minolta Sensing, Osaka, Japan) previously calibrated with CIE Standard Illuminant D65, a 8 mm-diameter aperture, and a 2 standard observer (AMSA, 2012). Samples were macerated to a thickness of 3 cm in a beaker with a diameter of 15 cm. Results are expressed in CIELAB L^* (lightness), a^* (redness) and b^* (yellowness) values. Measurements were performed in triplicate for each treatment.

2.5. Bacteriological analysis

Bacteriological analyses of the MSHM, MSCM and sausages were performed on day 0. Total aerobic mesophilic bacteria (TAMB), Coagulase-positive *Staphylococcus* bacterial counts, and most probable number of thermotolerant coliforms were evaluated. The presence of *Salmonella* spp. was evaluated and the results were expressed as presence or absence in 25 g of sample (APHA, 2001).

2.6. Sensory evaluation

The panelists were recruited from the students, faculty, and staff of the Universidade Federal Fluminense, Brazil. Sausage samples were analyzed at room temperature (20°C) and were coded with three-digit random numbers. The samples were presented to 100 untrained panelists (38 men and 62 women, 18 to 47 years old) who were instructed to evaluate their overall liking for each sausage sample, using the 9-point hedonic scale (1=dislike extremely, 9=like extremely). The panelists also recorded their purchase intent (5-point scale: 1 = "definitely would not buy", 5 = "definitely would buy") (STONE and SIDEL, 1998). These consumer tests were conducted in two stages: with no information (blind – first stage), and with the information "Mechanically separated tilapia meat was used to make the sausages, and its use reduces environmental pollution and encourages sustainability" (informed – second stage) (GARCIA *et al.*, 2009).

In the first stage, color, bitter taste, salty taste, succulence, elasticity and softness were also evaluated using a five-point Just About Right (JAR) scale (1 = much too weak, 2 =

somewhat weak, 3 = just about right, 4 = somewhat strong, and 5 = much too strong) according to CERVANTES *et al.* (2010).

An unsalted cracker and a glass of water at 25°C were offered to cleanse the palate between samples. The sensory evaluation was performed two days after the sausage was manufactured, to ensure adequate bacteriological quality.

2.7. Statistical analysis

Data for chemical composition, energy value, pH, overall liking and purchase intent were evaluated separately for treatment and time (days 0 and 34) or stages (blind and informed) by ANOVA. Cooking yield, instrumental color parameters, JAR data and bacteriological results were analyzed using one-way ANOVA. These data were further analyzed using a Tukey test when the means were considered different (P < 0.05). Chemical composition of MSHM and MSCM was evaluated by one-way ANOVA at a 95% confidence interval.

In addition, a principal components analysis (PCA) was performed to assess the parameters that were influenced by addition of inulin and potassium chloride. Partial least squares regression (PLSR) was performed to assess if the determinant parameters contributed positively or negatively to the overall liking of the samples. Penalty analysis (PA) was used to analyze the JAR data in order to identify possible alternatives for product improvement. Pearson's correlation at a 5% significance level (P < 0.05) was performed to correlate the physicochemical and sensory data (color and texture parameters). All statistical analyses were performed using the software XLSTAT version 2012.6.08 (Addinsoft, Paris, France).

3. RESULTS AND DISCUSSIONS

3.1. Physicochemical and bacteriological analyses of MSHM and MSCM

MSCM and MSHM exhibited 0.43% (±0.0006) and 0.73% (± 0.0008) ash, 86.14% (±0.001) and 69.41% (± 0.0069) water, 2.34% (±0.0013) and 18.39% (± 0.0112) lipid, and 4.78% (±0.0056) and 12.62% (± 0.0044) protein, respectively. The MSHM had a higher content of lipids, ash and proteins and lower water content than the MSCM. MONTEIRO *et al.* (2012) also observed higher lipid and ash, but lower water content in MSHM than in MSCM.

Regarding bacterial quality, *Salmonella* spp. and Coagulase-positive *Staphylococcus* were not detected in the samples. The most probable number of thermo-tolerant coliform and TAMB counts were within the official limits (ICMSF, 1986) which was also observed by MONTEIRO *et al.* (2012) in mechanically separated tilapia meat.

Our results suggest that the process of obtaining mechanically separated meat was conducted appropriately, and that the use of tilapia wastes can be a viable alternative for the development of value-added products.

3.2. Physicochemical parameters of tilapia sausage

Water, ash and protein contents did not differ significantly among the treatments (P > 0.05) on both days 0 and 34 (Table 2). T1 and T3 showed higher lipid and lower carbohydrate contents than T2 and T4 (P < 0.05). This can be explained by inulin addition, replacing the MSHM in T2 and T4. In agreement with our findings, MENEGAS *et al.* (2013) reported that foods with added carbohydrate (inulin) as a fat replacer showed lower lipid and higher carbohydrate contents.

Although the lipid content of T1 and T3 differed from T2 and T4, no difference (P > 0.05) was observed in energy value among all treatments, regardless of storage period. Thus, in the sausages with lower fat content, the loss of energy value was offset by the increased carbohydrate levels. However, sausages made with mechanically separated tilapia meat residue still have a very low caloric value, similar to low-fat sodium reduced fresh merguez sausage (TRIKI *et al.*, 2013). This is a desirable factor for both fish processors and consumers.

The pH values of the sausages did not differ (P > 0.05) among the treatments on days 0 and 34 of refrigerated storage. MONTEIRO *et al.* (2014c) also observed no difference among pH values of restructured tilapia steaks.

The samples were stored under refrigeration, and after 34 days of storage at 4 ± 1 °C, the analyses were repeated. No significant differences (P > 0.05) were observed in water, ash, protein, lipid, carbohydrate, energy value and pH values (Table 2), which indicates that the identity of the product was maintained after refrigerated storage.

Table 2: Chemical composition,	energy value and pH of	sausages made from	mechanically separated	d tilapia
meat.		-		_

Demonsterne	Storage	Treatments*						
Parameters	time (days)	T1	Т2	Т3	T4			
Water	0	74.05 ^{ax} ±1.76	70.28 ^{ax} ±2.09	73.33 ^{ax} ±3.89	70.22 ^{ax} ±1.50			
	34	72.86 ^{ax} ±2.69	69.30 ^{ax} ±3.07	73.43 ^{ax} ±2.66	70.02 ^{ax} ±2.53			
Ash	0	2.99 ^{ax} ±0.51	2.92 ^{ax} ±0.52	3.06 ^{ax} ±0.52	3.02 ^{ax} ±0.38			
	34	3.05 ^{ax} ±0.51	2.94 ^{ax} ±0.46	3.03 ^{ax} ±0.36	2.93 ^{ax} ±0.42			
Protein	0	14.26 ^{ax} ±1.51	13.77 ^{ax} ±1.36	14.56 ^{ax} ±1.38	13.71 ^{ax} ±1.00			
	34	12.61 ^{ax} ±1.37	12.51 ^{ax} ±2.09	14.04 ^{ax} ±0.70	14.33 ^{ax} ±1.29			
Lipid	0	2.64 ^{ax} ±0.06	0.86 ^{bx} ±0.15	3.16 ^{ax} ±0.09	1.38 ^{bx} ±0.49			
	34	3.82 ^{ax} ±1.24	1.72 ^{bx} ±1.05	3.02 ^{ax} ±0.39	2.02 ^{bx} ±0.82			
Carbohydrate	0	6.06 ^{bx} ±2.45	12.18 ^{ax} ±2.70	5.90 ^{bx} ±2.09	11.68 ^{ax} ±0.82			
	34	7.66 ^{bx} ±2.33	13.54 ^{ax} ±1.84	6.48 ^{bx} ±2.18	10.71 ^{ax} ±0.82			
Energy volue	0	105.05 ^{ax} ±5.21	111.52 ^{ax} ±6.17	110.24 ^{ax} ±13.14	113.12 ^{ax} ±6.63			
Energy value	34	115.44 ^{ax} ±14.84	119.68 ^{ax} ±15.48	109.28 ^{ax} ±10.80	116.55 ^{ax} ±6.86			
рН	0	6.72 ^{ax} ±0.02	6.71 ^{ax} ±0.08	6.75 ^{ax} ±0.04	6.76 ^{ax} ±0.01			
	34	6.68 ^{ax} ±0.10	6.73 ^{ax} ±0.01	6.72 ^{ax} ±0.06	6.71 ^{ax} ±0.06			

* T1 (100% NaCl without inulin), T2 (100% NaCl + 6% inulin), T3 (50% NaCl and 50% KCl + without inulin), T4 (50% NaCl and 50% KCl + 6% inulin).

Water, ash, protein, lipid and carbohydrate expressed in g/100g of sample.

Energy value expressed in kcal/100g.

Values are means±SD.

→ Means in a row without common superscripts are different (P < 0.05); n = 2.

• Means in a column with common superscripts did not exhibit difference (P > 0.05); n = 2.

With regard to cooking yield, no differences (P > 0.05) were observed among the treatments (Table 3). Similarly, HORITA *et al.* (2011) and MONTEIRO *et al.* (2014b) found no significant differences in cooking yield when NaCl was replaced with KCl in low-fat

bologna and restructured tilapia steaks, respectively. However, TRIKI *et al.* (2013) reported that replacing sodium chloride with a salt mixture (potassium chloride, calcium chloride and magnesium chloride) increased cooking losses.

The direct relationship between fat content and cooking yield is due to the emulsion stability provided by fat. Inulin has the ability to form a gel and acts similarly to fat (FRANCK, 2002). In this study, there was no relationship between fat content and cooking yield. Similarly, BRENNAN *et al.* (2004) found no differences in cooking loss (fluid loss) with the inclusion of inulin in spaghetti pasta. However, ÁLVAREZ and BARBUT (2013) suggested that inulin powder produces a higher yield compared to inulin gel. FELISBERTO *et al.* (2015) suggested that significant fluid losses were observed in formulations containing inulin or polydextrose. Our results suggest that inulin and KCl addition at the levels of the present study did not affect the product yield.

Color from tilapia muscle can be strongly affected by meat processing, and consequently influence consumer acceptability. Therefore, it is important to study color behavior in fish waste destined for human consumption, to predict changes that may occur in the final product (RAWDKUEN et al., 2009; MONTEIRO et al., 2014a). T4 exhibited a higher L* value (P < 0.05) than the other treatments (Table 3). MENEGAS *et al.* (2013) observed that the inclusion of inulin increased the lightness of dry-fermented chicken sausage. In addition, MONTEIRO et al. (2014b) observed that the L* value was intensified in restructured tilapia steaks manufactured with 50% NaCl and 50% KCl. T2 and T4 presented higher a^* values (P < 0.05). These formulations had a lower lipid content (inulin added to replace MSHM). CANDOGAN and KOLSARICI (2003) noted that products with reduced lipid tend to be redder, due to concentration of lean meat, which in this study, was represented by the MSCM. T3 exhibited lower (P < 0.05) a^* and b^* values than the other treatments (Table 3), suggesting that replacing 50% NaCl with KCl can affect color parameters, as also observed by MONTEIRO et al. (2014b) in tilapia products. In contrast to our findings, HORITA et al. (2011) and CANTO et al. (2014) found no difference (P >0.05) in the *a*^{*} and *b*^{*} values in reduced-fat mortadella and restructured caiman steaks after replacing 50% NaCl with KCl, respectively. Nevertheless, it is important to evaluate if the differences detected with instrumental analyses will be perceived by consumers.

Physicochemical	Treatments*							
parameters	T1	T2	Т3	Τ4				
Cooking yield (%)	78.48 ^a ±0.08	82.67 ^a ±0.04	77.75 ^a ±0.04	77.07 ^a ±0.03				
L*	63.31 ^b ±3.96	63.80 ^b ±3.45	63.97 ^b ±2.41	67.20 ^a ±4.21				
a*	16.57 ^b ±0.67	18.15 ^a ±0.60	16.13 ^c ±0.42	18.53 ^ª ±0.14				
b*	6.85 ^{ab} ±0.15	7.27 ^a ±0.70	6.44 ^b ±0.08	7.35 ^a ±1.23				

Table 3: Physicochemical parameters of sausages manufactured from mechanically separated tilapia meat.

*T1 (100% NaCl without inulin), T2 (100% NaCl + 6% inulin), T3 (50% NaCl and 50% KCl + without inulin), T4 (50% NaCl and 50% KCl + 6% inulin).

 L^* ranges from 0 (black) to 100 (white); a^* ranges from red (+ a^*) to green (- a^*); and b^* ranges from yellow (+ b^*) to blue (- b^*).

Values are means±SD.

~ Means in a row without common superscripts are different (P < 0.05); n = 2.

3.3. Bacteriological quality of tilapia sausages

No difference (P > 0.05) was observed among treatments. TAMB counts were below the limit of 7.0 log CFU/g (ICMSF, 1986) in all treatments, ranging from 3.06 to 3.38 log

CFU/g. *Salmonella* sp., Coagulase-positive *Staphylococcus* and thermo-tolerant coliforms were not detected in any sausage formulation.

In the present study, the partial replacement of salts and inulin addition did not compromise the bacteriological quality of the product. DESMOND (2006) and MENEGAS *et al.* (2013) reported similar results. Nevertheless, the adoption of hygienic procedures before, during, and after processing is essential to obtain a product with optimum quality. Our findings suggest that the sausage-making process was conducted appropriately.

3.4. Sensory evaluation of tilapia sausages

With regard to overall liking, all treatments exhibited mean scores between "like slightly" and "like moderately" (Table 4). OLIVEIRA FILHO *et al.* (2010) found lower overall liking for sausages with different percentages of inclusion of minced tilapia. In this study, differences were observed among the blind samples, where T2 showed higher (P < 0.05) overall liking than T3. After the scorers received information about the products, T3 and T4 exhibited lower (P < 0.05) overall liking than T1 and T2. All formulations received mean scores between "maybe/maybe not" and "probably would buy" (Table 4), which indicates the consumer acceptability of this new product. In general, T1 and T2 showed higher scores (P < 0.05) for purchase intent than T3 and T4. The sentence "Mechanically separated tilapia meat was used to make the sausages, and its use reduces environmental pollution and encourages sustainability" submitted to the panelists did not affect (P > 0.05) the overall liking and purchase intent of all treatments. However, our results indicate that the inulin formulations had better potential, regardless of any information provided. MENEGAS *et al.* (2013) reported that acceptability of dry-fermented chicken sausages was not affected by inulin addition.

According to FRANCK (2002), when thoroughly mixed with water or another aqueous liquid, inulin forms a particle-gel network, resulting in a white creamy structure with a spreadable texture. This formulation can easily be incorporated into foods to replace up to 100% of the fat.

On the other hand, our findings for overall liking and purchase intent indicated that sodium reduction can be still a major challenge for the industry. Nevertheless, all formulations exhibited color, taste (bitter and salty), and texture (succulence, elasticity, and softness) attributes close to ideal (2.51 - 3.55) (Table 4). Color, salty taste, succulence and elasticity did not differ (P > 0.05) among treatments, suggesting that the consumers were unable to differentiate the low-sodium and inulin added in the formulations by these attributes. T3 showed a stronger (P < 0.05) bitter taste than T2, whereas softness was lower (P < 0.05) in T1 compared to the T4 formulation.

ARMENTEROS *et al.* (2012) noted that reduction of the salt content by more than 40–50% negatively affected the sensory quality of ham, especially taste, with some bitter and metallic after tastes perceived by consumers.

In agreement with our results, PALMEIRA *et al.* (2014a) observed taste (spicy and bitter) and texture attributes close to ideal in trout meatballs with salt replacement; however, the consumers perceived difference among formulations by these attributes. MONTEIRO *et al.* (2014b) found close to ideal taste (bitter and salty) and texture attributes in restructured tilapia steaks manufactured with 50% salt replacer (KCl). CANTO *et al.* (2014) found a salty taste and texture attributes close to ideal in restructured caiman steaks with 50% KCl. Both authors reported that consumers were not able to differentiate among treatments with respect to attributes evaluated.

Table 4: Sensory evaluation of sausages made from mechanically separated tilapia meat.

Overall liking		Treatn	nents*	
	T1	T2	Т3	Τ4
Blind	6.61 ^{abx} ±1.41	6.79 ^{ax} ±1.51	6.17 ^{bx} ±1.52	6.33 ^{abx} ±1.63
Informed	6.93 ^{ax} ±1.35	6.80 ^{ax} ±1.58	6.08 ^{bx} ±1.72	6.20 ^{bx} 1.69
Purchase intent	T1	T2	Т3	T4
Blind	3.51 ^{abx} ±0.93	3.57 ^{ax} ±1.15	3.16 ^{bx} ±1.01	3.17 ^{bx} ±1.04
Informed	3.68 ^{abx} ±1.00	3.76 ^{ax} ±1.10	3.17 ^{cx} ±1.26	3.28 ^{bcx} ±1.15
JAR attributes	T1	T2	Т3	Τ4
Color	2.74 ^a ±0.79	2.55 ^a ±0.76	2.77 ^a ±0.96	2.51 ^ª ±0.75
Bitter taste	3.10 ^{ab} ±0.54	2.91 ^b ±0.60	3.18 ^a ±0.66	3.07 ^{ab} ±0.70
Salty taste	$3.00^{a} \pm 0.55$	$3.00^{a} \pm 0.65$	2.88 ^a ±0.73	2.85 ^ª ±0.69
Succulence	2.90 ^a ±0.75	2.96 ^a ±0.67	2.89 ^a ±0.84	2.92 ^a ±0.88
Elasticity	2.79 ^a ±0.74	2.73 ^a ±0.83	2.68 ^a ±0.91	2.69 ^a ±0.90
Softness	3.17 ^b ±0.83	3.38 ^{ab} ±0.84	3.40 ^{ab} ±0.91	3.55 ^ª ±0.85

*T1 (100% NaCl without inulin), T2 (100% NaCl + 6% inulin), T3 (50% NaCl and 50% KCl + without inulin), T4 (50% NaCl and 50% KCl + 6% inulin).

Blind - with no information; and informed - "Mechanically separated tilapia meat was used to make the sausages, and its use reduces environmental pollution and encourages sustainability".

Values are means±SD.

 \sim Means in a row without common superscripts are different (*P* < 0.05).

• Means in a column with common superscripts did not exhibit difference (P > 0.05).

Two principal components (PC1 and PC2) explained 99.73% of total data variance (Fig. 1) and separated two groups (T1 and T3; T2 and T4), based mainly on lipid and carbohydrate contents, bitter taste, texture parameters, and *a** and *b** values. The inulin treatments (T2 and T4) were characterized by greater softness, succulence, carbohydrate, *a** and *b** values, with a less bitter taste and lower lipid content than T1 and T3. Taste and texture attributes were the most important for the salt-replacer formulations. T3 showed a stronger bitter taste and less softness than T4. MONTEIRO *et al.* (2014b) found that replacing 50% of NaCl with KCl slightly increased the bitter taste and negatively influenced the succulence and softness. MENDOZA *et al.* (2001) found a softer texture in cooked meat products when the inulin was added. Our results suggest that adding inulin can minimize the negative effects of KCl on the sensory parameters of food products.

The most important correlations were between softness and succulence (r = 1.00), between softness and elasticity (r = 1.00), between cooking yield and bitter taste (r = -0.99), between cooking yield and salty taste (r = -0.99), between lipid and carbohydrate (r = -0.92) and between lipid and a^* values (r = -0.85). These correlations may explain the stronger bitter taste perceived in the formulations with salt replacer (T3 and T4), which was probably influenced by cooking yield. The direct relationship between softness and succulence as well as the inverse relationship between lipid, carbohydrate and a^* values may clarify the positive effect of inulin addition on the chemical composition, color and texture parameters observed.



T1 (100% NaCl without inulin), T2 (100% NaCl + 6% inulin), T3 (50% NaCl and 50% KCl + without inulin), T4 (50% NaCl and 50% KCl + 6% inulin).

Figure 1: Physicochemical and sensory data of sausages made from mechanically separated tilapia meat defined by two principal components.

The PLSR model (Fig. 2) explained 99.4% of consumer acceptance (Y-axis) and 93.1% of the untrained-panel sensory scores and physicochemical parameters (X-axis), with a cumulative Q² of 0.925. The X-axis parameters were considered important when their respective 'Variable Important to the Projection' was > 1.0 (WOLD *et al.*, 2001). Ash, pH and bitter taste were detrimental to overall liking whereas cooking yield, salty taste, succulence and elasticity positively contributed to overall liking (Fig. 3). With regard to cooking yield, T3 and T4 showed similar results, as did T1 and T2. This can be explained by the greater ionic strength of potassium (1.33) than sodium (0.95), which decreases the electrostatic repulsion between the peptide chains and the space between the myofibrils, retaining less water between the myofibrillar spaces (MARCHESE and BEVERIDGE, 1984; DAMODARAN *et al.*, 2007).

Regarding to Penalty Analysis, major detrimental attributes were those with a > 0.5 penalty score and more that 20% occurrence. T1, T2 and T3 were penalized as having too-weak color (Table 5). Color intensity (a^* and b^*) was lower in T1 and T3 than T2 and T4, suggesting that the panelists were unable to differentiate color among treatments. Moreover, color was not determinant for acceptability. Only formulation T3 was penalized for having a too-bitter taste. The consumers penalized the T3 and T4 formulations as having a too-weak salty taste. This results suggest that use of 50% KCl can decrease the salt perception, which was important for product acceptability. Regarding the texture attributes, T1, T3 and T4 were penalized as having too little succulence, and T1 and T3 as having too little elasticity. Moreover, only formulation T1 was penalized for being too soft.



X axis = physicochemical parameters; Y axis = overall liking. T1 (100% NaCl without inulin), T2 (100% NaCl + 6% inulin), T3 (50% NaCl and 50% KCl + without inulin), T4 (50% NaCl and 50% KCl + 6% inulin).

Figure 2: Partial Least Square regression model for sensory attributes and physicochemical parameters of sausages made from mechanically separated tilapia meat.



*CY – Cooking yield.

Figure 3: Weighted regression coefficients of physicochemical and sensory parameters detrimental to acceptability by partial least squares regression.

Apparently, KCl affected texture parameters that were determinant for acceptability, whereas inulin addition to foods together with sodium reduction may be an alternative to improve the acceptability of these products, taking into account the enhancement of taste and texture.

The effect of fiber addition in products differs, depending on the type and the level of the fiber added, as well as by the presence of other ingredients (JIMENEZ-COLMENERO *et al.*, 2005).

Table 5: Consumer	penalty	analysis	of the J.	AR	diagnostic	attributes	(percentage of	consumers	and me	ean
decreases).					-					

Treatments	Color		Bitte	r taste	Salty taste		
	Too weak	Too strong	Too weak	Too strong	Too weak	Too strong	
T1	35.0 [*] (0.90) [#]	-	-	-	-	-	
T2	45.0 (1.01)	-	-	-	-	-	
ТЗ	36.0 (0.53)	-	-	29.0 (0.83)	27.0 (0.36)	-	
T4	-	-	-	-	29.0 (0.77)	-	
Treatments	Succu	lence	Elasticity		Softness		
	Too weak	Too strong	Too weak	Too strong	Too weak	Too strong	
T1	27.0 (1.22)	-	33.0 (0.75)	-	-	31.0 (0.57)	
Т2	-	-	-	-	-	-	
ТЗ	32.0 (1.04)	-	42.0 (0.73)	-	-		
T4	34.0 (1.44)	-		-	-		

T1 (100% NaCl without inulin), T2 (100% NaCl + 6% inulin), T3 (50% NaCl and 50% KCl + without inulin), T4 (50% NaCl and 50% KCl + 6% inulin).

* The percentage of consumers who found treatments to be too weak or too strong for JAR color, bitter taste, salty taste, succulence, elasticity, and softness.

The number in parentheses is the change in mean compared to the consumer response score to overall liking.

(-) indicates that less than 20% of consumers choose that JAR category.

Our findings indicate that the level of inulin used was sufficient to maintain the physicochemical and sensory parameters of the tilapia sausages. On the other hand, 50% KCl as a sodium-chloride replacer negatively influenced the sensory attributes. Further studies should be performed to evaluate lower levels of KCl and/or the use of other ingredients in this product since herbs and spices is a promising alternative to suppress or decrease the sensory effects caused by the use of KCl (AHN *et al.*, 2004).

4. CONCLUSIONS

Sausages manufactured with mechanically separated tilapia meat represent a potential alternative for sustainable use of this waste, with high consumer acceptance. The inclusion of inulin is an option to produce a low-fat food, improve the emulsion stability, and ensure the prebiotic effect of the sausage. Replacing sodium with 50% KCl decreased the

acceptance and purchase intent of the tilapia sausages; however, the inclusion of inulin in fish products with sodium reduction is a promising technological strategy to solve possible problems with taste and texture due to KCl addition.

ACKNOWLEDGEMENTS

The authors are grateful for financial support from the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), process numbers FAPERJ E-26/112.620/2012, E-26/111.130/2014 and E-26/101.403/2014; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), process numbers CNPq 401922/2013-8 and 441987/2014-1; and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)/FAPERJ E-45 - PAPDRJ/2013 (E-26/101.403/2014). D.P. Bessa was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). The Centro de Tecnologia (SENAI) de Alimentos e Bebidas is gratefully acknowledged for providing the processing-plant facilities.

REFERENCES

Ahn J., Grün I.U. and Mustapha A. 2004. Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. J. Food Protect. 67:148-155.

Álvarez D. and Barbut S. 2013. Effect of inulin, β -Glucan and their mixtures on emulsion stability, color and textural parameters of cooked meat batters. Meat Sci. 94:320-327.

AMSA. 2012. "Meat color measurement guidelines" 1st Ed. American Meat Science Association, Champaign, USA.

AOAC. 2012. "Official Methods for analysis" 19^a Ed. Association of Official Analytical Chemists, Gaithersburg, USA.

APHA. 2001. "Compendium of methods for the microbiological examination of foods" 4^a Ed. American Public Health Association, Washington, DC.

Armenteros M., Aristoy M.C., Barat J.M. and Toldrá F. 2012. Biochemical and sensory changes in dry-cured ham salted with partial replacement of sodium by a mixture of potassium, calcium and magnesium. Meat Sci. 90:361-367.

Brennan C.S., Kuri V. and Tudorica C.M. 2004. Inulin-enriched pasta: effects on textural properties and starch degradation. Food Chem. 86:189-193.

Candogan K. and Kolsarici N. 2003. Storage stability of low-fat beef frankfurters formulated with carrageenan or carrageenan with pectin. Meat Sci. 64:207-214.

Canto A.C.V.C.S., Costa Lima B.R.C., Suman S.P., Lazaro C.A., Monteiro M.L.G., Conte-Junior C.A., Freitas M.Q., Cruz A.G., Santos E.B. and Silva T.J.P. 2014. Physico-chemical and sensory attributes of low-sodium restructured caiman steaks containing microbial transglutaminase and salt replacers. Meat Sci. 96:623-632.

Cervantes B.G., Aoki N.A. and Almeida C.P.M. 2010. Aceitação sensorial de biscoito de polvilho elaborado com farinha de okara e análise de dados com metodologia de penalty analysis. Braz. J. Food Technol. 1: 3-10.

Conte-Júnior C.A., Fernández M. and Mano S.B. 2008. Use of carbon dioxide to control the microbial spoilage of bullfrog (*Rana catesbeiana*) meat. Ch. 3. In "Modern Multidisciplinary Applied Microbiology". A. Mendez-Vilas (Ed.) p. 356-361. Wiley-VCH Verlag GmbH & Co. KGaA, Germany, DE.

Damodaran S., Parkin K.L. and Fennema O.R. (Ed.). 2007. "Fennemas food chemistry" 4th ed. CRC Press, Boca Raton, Fl.

Deckelbaum R.J. and Torrejon C. 2012. The omega-3 fatty acid nutritional landscape: Health benefits and sources. J. Nutr. 142:587S-591S.

Desmond E. 2006. Reducing salt: A challenge for the meat industry. Meat Sci. 74:188-196.

Dotsch M., Busch J., Batenburg M., Liem G., Tareilus E., Mueller R. and Meijer G. 2009. Strategies to reduce sodium consumption: A food industry perspective. Food Sci. Nutr. 49:841-851.

Felisberto M.H.F., Galvão M.T.E.L., Picone C.S.F., Cunha R. and Pollonio M.A.R. 2015. Effect of prebiotic ingredients on the rheological properties and microstructure of reduced-sodium and low-fat meat emulsions. LWT - Food Sci. Technol. 60:148-155.

Franck A. 2002. Technological functionality of inulin and oligofructose. Brit. J. Nutr. 87:S287-S291.

Garcia K., Sriwattana S., No H.K., Corredor J.A.H. and Prinyawiwatkul W. 2009. Sensory optimization of a mayonnaise-Type spread made with rice bran oil and soy protein. J. Food Sci. 74:248-254.

Horita C.N., Morgano M.A., Celeghini R.M.S. and Pollonio M.A.R. 2011. Physico-chemical and sensory properties of reduced-fat mortadella prepared with blends of calcium, magnesium and potassium chloride as partial substitutes for sodium chloride. Meat Sci. 89:426-433.

ICMSF. 1986. "Microorganisms in foods, sampling for microbiological analysis: principles and specific applications" 2^{ad} Ed. International Commission on Microbiological Specifications for Foods, Toronto, Canada.

Jimenez-Colmenero F., Ayo M.J. and Carballo J. 2005. Physicochemical properties of low sodium frankfurter with added walnut: effect of transglutaminase combined with caseinate, KCl and dietary fiber as salt replacers. Meat Sci. 69:781-788.

Marchese F.T. and Beveridge D.L. 1984. Pattern recognition approach to the analysis of geometrical features of solvation: application to the aqueous hydration of lithium(1+), sodium(1+), potassium(1+), fluoride(1-), and chloride(1-) ions. J. Am. Chem. Soc. 106:3713-3720.

Mendoza E., García M.L., Casas C. and Selgas M.D. 2001. Inulin as fat substitute in low fat, dry fermented sausages. Meat Sci. 57:387-393.

Menegas L.Z., Pimentel T.C., Garcia S. and Prudencio S.H. 2013. Dry-fermented chicken sausage produced with inulin and corn oil: Physicochemical, microbiological, and textural characteristics and acceptability during storage. Meat Sci. 93:501-506.

Montanhini Neto R. and Ostrensky A. 2013. Nutrient load estimation in the waste of Nile tilapia *Oreochromis niloticus* (L.) reared in cages in tropical climate conditions. Aquac. Res. In press.

Monteiro M.L.G., Mársico E.T., Viriato I.M., Souza J.M.L. and Conte Junior C.A. 2012. Preparation of added value by products from the waste material of tilapia (*Oreochromis niloticus*) processing. J. Aquac. Res. Dev. 3:150-155.

Monteiro M.L.G., Mársico E.T., Lázaro C.A., Ribeiro R.O.R., Jesus R.S. and Conte-Júnior C.A. 2014a. Flours and instant soup from tilapia wastes as healthy alternatives to the food industry. Food Sci. Technol. Res. 20:571-581.

Monteiro M.L.G., Mársico E.T., Lázaro C.A., Canto A.C.V.C.S., Costa Lima B.R.C., Cruz A.G. and Conte-Junior C.A. 2014b. Partial sodium replacement in tilapia steak without loss of acceptability. Food Sci. Technol. Int. In press.

Monteiro M.L.G., Mársico E.T., Lázaro C.A., Canto A.C.V.C.S., Costa Lima B.R.C., Cruz A.G. and Conte-Júnior C.A. 2014c. Effect of transglutaminase on quality characteristics of a value-added product tilapia wastes. J. Food Sci. Technol. 52: 2598-2609.

Oliveira Filho P.R.C., Netto F.M., Ramos K.K., Trindade M.A. and Viegas E.M.M. 2010. Elaboration of sausage using minced fish of Nile tilapia filleting waste. Braz. Arch. Biol. Technol. 53:1383-1391.

Palmeira K.R., Rodrigues B.L., Gaze L., Freitas M.Q., Teixeira C.E., Marsico E.T., Cruz A.G. and Conte Junior C.A. 2014a. Use of transglutaminase, soybean waste and salt replacement in the elaboration of trout (*Oncorhynchus mykiss*) meatball. Int. Food Res. J. 21:1597-1602.

Palmeira K.R., Mársico E.T., Doro L.H., Lemos M., Teixeira C.E., Paschoalin V.M.F., Monteiro M.L.G. and Conte-Junior C.A. 2014b. Quality of semi-prepared products from rainbow trout waste (*Onchorynchus mykiss*) by using different technological strategics. Food Nutr. Sci. 05:571-580.

Rawdkuen S., Sai-Ut S., Khamsorn S., Chaijan M. and Benjakul S. 2009. Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process. Food Chem. 112: 112-119.

Stone H. and Sidel J.L. 1998. Quantitative descriptive analysis: developments, applications, and the future. Food Technol. 52:48-52.

Triki M., Herrero A.M., Jiménez-Colmenero F. and Ruiz-Capillas C. 2013. Storage stability of low-fat sodium reduced fresh merguez sausage prepared with olive oil in konjac gel matrix. Meat Sci. 94:438-446.

Wold S., Sjostrom M. and Eriksson L. 2001. PLS-Regression: A Basic tool of chemometrics. Chemometr. Intell. Lab. 58:109-130.

Paper Received May 19, 2015 Accepted August 20, 2015