Fat substitutes in processing of sausages using piramutaba waste

Lúcia de Fátima Henriques Lourenço ·
Giane Célia dos Santos Galvão ·
Suezil de Conceição Amaral Ribeiro ·
Carmelita de Fátima Amaral Ribeiro · Kil Jin Park

Revised: 28 January 2012 / Accepted: 3 February 2012 / Published online: 23 February 2012
© Association of Food Scientists & Technologists (India) 2012

Abstract The aim of this study was to evaluate fat substitute in processing of sausages prepared with surimi of waste from piramutaba filleting. The formulation ingredients were mixed with the fat substitutes added according to a fractional planning 2^{4-1} , where the independent variables, manioc starch (Ms), hydrogenated soy fat (F), texturized soybean protein (Tsp) and carrageenan (Cg) were evaluated on the responses of pH, texture (Tx), raw batter stability (RBS) and water holding capacity (WHC) of the sausage. Fat substitutes were evaluated in 11 formulations and the results showed that the greatest effects on the responses were found to Ms, F and Cg, being eliminated from the formulation Tsp. To find the best formulation for processing piramutaba sausage was made a complete factorial planning of 2^3 to evaluate the concentrations of fat substitutes in an enlarged range. The optimum condition found

for fat substitutes in the sausages formulation were carrageenan (0.51%), manioc starch (1.45%) and fat (1.2%).

Keywords Microbiological · Surimi · Carrageenan · Fat Replacement · Manioc Starch

Introduction

The piramutaba (*Brachyplatystoma vaillantii*) belonging to the Pimelodidae family of Siluriformes order is a species of freshwater fish more caught in the Amazon being very important to the regional, national and international economy. Over 30,000 t of this species are sold annually in the Amazon (Fabr and Barthm 2005; MPA 2009).

Piramutaba fishing is the second in economic importance to the Northern region, whereas surimi only losing to shrimp. Nationally, historically, has been occupying the third place in exportations. Considering the social aspects, the piramutaba, tambaqui and jaraqui fisheries are most representative for that region, whether by labor or by fish volume generated for domestic consumption (Fabr and Barthm 2005).

Surimi is a paste of fish meat frozen tasteless, odorless, highly viscous and elastic, but unstable to freezing in the absence of cryoprotectives such as sorbitol and glucose, mainly made of white muscle crushed, washed, drained and stabilized by the addition of cryoprotectives (Auh et al. 2003). In addition carbohydrates, in particular sucrose was reported to inhibit the formation of biogenic amines in dry cured fish at a low storage temperature (4 °C) (Zhang et al. 2011).

Surimi is used as raw material in the preparation of traditional food of Japanese cuisine, such as “kamaboko”, and has been used also for production of similar products of seafood such as shrimp, lobster, scallops, or the traditional “kani kama”—crab and crab analogs, compounds emulsified

L. de Ftima Henriques Loureno (✉) · G. C. dos Santos Galvo
Universidade Federal do Par - Rua Augusto Corra,
01 - Guam,
CEP 66075-110 Belm, PA, Brazil
e-mail: luciah1@ufpa.br

S. da Conceio Amaral Ribeiro
Federal Office for Education, Science and Technology of Par,
IFPA, Br. 316, KM 62,
CEP: 687409-70 Castanhal, PA, Brazil
e-mail: suzi@eafc-pa.gov.br

S. da Conceio Amaral Ribeiro · C. de Ftima Amaral Ribeiro
Center for Natural Science and Technology,
State University of Par, UEPA,
Trav. Enas Pinheiro, Marco, P. O. Box 2626, CEP: 66095-100
Belm, PA, Brazil

K. J. Park
Faculty of Agricultural Engineering, State University of Campinas,
UNICAMP,
P. O. Box 6011, CEP: 13083-875 Campinas, SP, Brazil
e-mail: kil@agr.unicamp.br

highlighting ham and fish sausage (Park and Morrissey 2000; Chen 2000; Siddaiah et al. 2001; Barreto and Beirão 1999; Gonçalves et al. 2009). According to Mehta et al. (2011) the primary requirement for preparation of these products is the gel forming ability of the mince.

In this sense, the emulsified products offer great perspectives due to the ease of performing blends with different ingredients in order to improve and modify the sensory characteristics such as taste, odor, color and texture (Hughes et al. 1997; Santiago et al. 2004; Andrès et al. 2006; Oliveira Filho et al. 2010).

According to Olivo and Shimokomaki (2006), meat emulsions constitute a system of two phases; the dispersed phase consists of particles of solid or liquid fat and the continuous phase by water containing dissolved and suspended salts and proteins. These two phases, although they are immiscible, are stabilized and kept in peaceful coexistence, due to the active participation of the stabilizers. If the meat emulsion is unstable, larger fat and water separation will occur during cooking (Su et al. 2000; Tornberg 2005).

The sausages by being emulsified products, are required to give some way to providing the subsequent processing, and protect it. It can be used an automated system to divide the product into portions and twist or tie the gut to sausage maker (Keeton 1994).

According Olivo and Shimokomaki (2006) the sausage is a sausage meat coming from an emulsion (thin mass) formed after the mixing of meat, additives and condiments, promoting an intimate mixture of fat globules with a protein and water array. The production of sausages requires a wide variety of meat, no meat ingredients, and each performing a specific function according to their property. Emulsion activity indicates the ability of the emulsion to hold liquid at the time of performance of the emulsion and it is influenced by the fat content of the emulsion (Bhattacharyya et al. 2007).

Other fish species have also been used for elaboration of sausage, such as the tilapia *Oreochromis niloticus* (Oliveira Filho et al. 2010), shrimp *Fenneropenaeus indicus* (Sachindra and Mahendrakar 2010), silver carp *Hypophthalmichthys molitrix* (Hu et al. 2008). However, no reports are available on the utilization of surimi from piramutaba waste in the elaboration of sausages.

Thus the aim of this study was the processing of sausages prepared with surimi of waste from piramutaba filleting generated by industries in the state of Para evaluating the influence of fat substitutes.

Material and methods

Waste of piramutaba

Wastes of industrial filleting of piramutaba (*Brachyplatystoma vaillantii*) were obtained in the fishery industry Campasa S.A

and Pesqueira Maguary, located in the county of Curuçá and Belém, Pará State, respectively.

Additives and ingredients

Three companies supplied additives and ingredients used in the preparation of sausages specialized in the industrialization of food: Duas Rodas Industrial Ltda, Brasil (cochineal powder, sodium tripolyphosphate, sodium erythorbate and condiments for sausage), Doremus Alimentos Ltda, Brasil (texturized soybean protein, carrageenan kappa) and of wrappings were purchased from Kraki Kienast & Kratschmer Ltda (Jaraguá do Sul, SC, Brasil).

Microbiological analysis

Microbiological determinations of piramutaba sausage (obtained in the final formulation) were performed at the Laboratory of Microbiology, Faculty of Food Engineering (FEA) of UFPA according to the standards required by legislation (Brasil 2001). Measurements of *coliforms* were performed at 45 °C, *Salmonella*, positive *coagulase Staphylococci*, *Clostridium sulfite*, standard *psychrotrophs* count, yeasts and molds count. All tests were performed using the methodology described by Vanderzant and Splittstoesser (1992).

Preparation of surimi

Preparation of surimi was performed according to methodology described by Barreto and Beirão (1999), with some adjustments. Piramutaba wastes (frozen) were crushed into Master R17633 processor, Walitta. The mass obtained was washed (three cycles) with drinking water, at intervals of 10 min with slow manual shaking followed by a rest of 5 min for decanting. In the washing step, the first two washes were performed only with water and the third by adding 2% NaCl on the amount of pulp, to assist the extraction of water-soluble proteins. The ratio water: pulp was 5:1, and a portion of the water was ice to keep the temperature between 4 and 5 °C. Drainage was done using a centrifuge to remove the excess water. Subsequently added to the mass cryoprotectants, sodium tripolyphosphate (0.2%) and sorbitol (4%) and mixed in homogenizer cutter type (Filizzola) for 5 min. The surimi was divided into portions of 500 g in plastic bags, vacuum packed (FastVac, Model F200), frozen and stored at −22 °C.

Preparation of sausages

The surimi was thawed in running water and then mixed with other ingredients in the formulation of the sausage: surimi (70%), ice (20%), NaCl (1%), curing salt (0.2%),

condiments for sausage (1.5%), sodium tripolyphosphate (0.3%), sodium erythorbate (0.3%), monosodium glutamate (0.5%) and cochineal powder (0.02%).

All ingredients and surimi were crushed in the cutter (Fillizola, Sire, Brasil). The other ingredients that corresponded to the replacement of fats studied, manioc starch (Ms), texturized soybean protein (Tsp), hydrogenated soy fat (F), carrageenan (Cg) were added as the experimental planning (Table 1).

For the elaboration of sausage ingredients and surimi were added and mixed in the cutter, in the following order: 1) surimi, ¼ ice, texturized soybean protein and NaCl, for 3 min; 2) condiments for sausage, carrageenan and curing salt, for 5 min; 3) ¼ ice and hydrogenated soy fat, for 2 min 4) ¼ manioc starch and sodium erythorbate, for 1 min.

The sausage weight remained at rest for 30 min to allow the development of flavor and was later embedded in a manual sausage maker, using sausage casings of 21 mm diameter, pre-hydrated and separated into slices of 10 cm in length.

The heat treatment of the sausage was made in two steps, starting with a pre-cooking in an incubator with circulating air at 60 °C for 30 min, then raised to the temperature to 80 °C until the sausage reaches 72 °C internally with the aid of a thermocouple. The cooling was carried out with water bath and ice until the internal temperature of 40 °C. The process of membranes delamination was performed immediately after cooling products of piramutaba sausages were packed in polythene bags vacuum packed and stored under refrigeration (5±2 °C).

Experimental designs

To evaluate the significance and effects of the factors studied in the processing of piramutaba sausages held a 2⁴⁻¹ fractional experimental design, based on the best results, i.e., removing the variables not significant at 95% confidence, was elaborated a 2³ complete factorial design for model generation and response surface to determine the best process conditions. The process variables studied were manioc starch (Ms),

texturized soybean protein (Tsp), hydrogenated soy fat (F) and carrageenan (Cg). The results were evaluated by the responses of pH, texture, raw batter stability (RBS) and water holding capacity (WHC) of the sausage.

The 2⁴⁻¹ fractional experimental design was conducted with eight factorial points (+1, -1) and three central points (0) totaling 11 experiments. The not significant effects for the implementation of a full factorial design were determined and deleted.

The 2³ complete experimental design was conducted with eight factorial points (+1, -1), six axial points (-α=-1.68; +α=+1.68) and three central points (0) and were observed a good linear and quadratic correlation of experimental data in the processing of sausages.

Table 1 shows the levels of process variables used in the fractional experimental design 2⁴⁻¹ and the complete factorial design 2³ in the processing of piramutaba sausages.

The response surface methodology (RSM) was used to evaluate the effect of Ms, Tsp, F and Cg (independent variables) on the responses: pH, texture, raw batter stability (RBS) and water holding capacity (WHC), at the end of the process. The data were adjusted to the following polynomial (Eq. 1):

$$Y = \beta_0 + \beta_1 Ms + \beta_{11} Ms^2 + \beta_{2F} F + \beta_{22} F^2 + \beta_3 Cg + \beta_{33} Cg^2 + \beta_{12} MsF + \beta_{13} MsCg + \beta_{23} FCg \quad (1)$$

Where:

Y	response function
Ms, Tsp, F and Cg	Values of independent variables
β ₁ , β ₂ and β ₃	linear coefficients estimated by the least squares method
β ₁₁ , β ₂₂ and β ₃₃	coefficients of the quadratic variables
β ₁₂ , β ₁₃ and β ₂₃	coefficients of interaction between the independent variables.

Evaluation of parameters

Hydrogenionical potential (pH)

The pH of the sausage samples were measured with a potentiometer (Minolta brand).

Texture (Tx)

Measures of texture of piramutaba sausages were performed by the compression method, using the texture analyzer brand Rheometer (Rheo Tex SD – 700, Sun Scientific Co., Ltd, Japan).

Sausages emulsion stability (RBS)

The stability of the raw food (raw batter stability (RBS) of sausage piramutaba was determined according to the

Table 1 Process variables and levels used in designs for the processing of piramutaba sausages

Process variables	-α	-1	0	+1	+α
a) Fractional experimental design 2 ⁴⁻¹					
Manioc starch (Ms)%	–	1	1.5	2	–
Texturized soybean protein (Tsp)%	–	0.5	0.75	1	–
Content of hydrogenated soy fat (F)%	–	1	1.5	2	–
Carrageenan (Cg)%	–	0.25	0.5	0.75	–
b) Complete factorial design 2 ³					
Manioc starch (Ms)%	0.66	1	1.5	2	2.34
Content of hydrogenated soy fat (F)%	0.66	1	1.5	2	2.34
Carrageenan (Cg)%	0.08	0.25	0.5	0.75	0.92

Table 2 Values of the levels of the variables in the fractional design and the responses of pH, Tx, RBS and WHC

Testing	Ms (%)	Tsp (%)	F (%)	Cg (%)	pH	Tx (g)	RBS (%)	WHC
1	1 (−1)	0.5 (−1)	1 (−1)	0.25 (−1)	7.13	388.75	88.16	0.46
2	2 (+1)	0.5 (−1)	1 (−1)	0.75 (+1)	7.11	317.66	89.11	0.35
3	1 (−1)	1 (+1)	1 (−1)	0.75 (+1)	7.09	305.00	89.62	0.39
4	2 (+1)	1 (+1)	1 (−1)	0.25 (−1)	7.05	345.00	89.99	0.35
5	1 (−1)	0.5 (−1)	2 (+1)	0.75 (+1)	7.06	327.30	89.38	0.38
6	2 (+1)	0.5 (−1)	2 (+1)	0.25 (−1)	7.04	315.50	88.58	0.32
7	1 (−1)	1 (+1)	2 (+1)	0.25 (−1)	7.08	308.00	88.39	0.30
8	2 (+1)	1 (+1)	2 (+1)	0.75 (+1)	6.97	175.00	90.98	0.45
9	1.5 (0)	0.75 (0)	1.5 (0)	0.5 (0)	7.09	82.00	80.37	0.49
10	1.5 (0)	0.75 (0)	1.5 (0)	0.5 (0)	7.10	124.00	85.52	0.48
11	1.5 (0)	0.75 (0)	1.5 (0)	0.5 (0)	7.07	106.00	84.58	0.48

Ms manioc starch; Tsp texturized soybean protein; F hydrogenated soy fat; Cg carrageenan; Tx texture; RBS raw batter stability; WHC water holding capacity
n=3

method of Hermansson (1980), described by Ambrosiadis et al. (1996) with some modifications.

Water holding capacity (WHC)

The method of pressure on filter paper as described by Zayas and Lin (1989) and adapted by Viana et al. (2003) was used to find the water holding capacity of the samples.

Statistical analysis

For the analysis of experimental data obtained in the preparation of sausage, we used the STATISTICA 5.0 program. In fractional design only was rated the intensity of the effects of independent variables using 95% significance.

For the analysis of experimental data obtained in the complete design, was considered predictive model that showed significant regression and lack of fit no significant with confidence of 90% and a high coefficient of determination R^2 . Each analytical determination was performed in triplicate ($n=3$)

Results and discussion

Effects of fat substitutes

Table 2 represented the values of pH, texture (Tx), raw batter stability (RBS) and water holding capacity (WHC) of sausages using the manioc starch, texturized soybean protein, hydrogenated soy fat and carrageenan, according to the fractional design worksheet.

The results of statistical analysis, applied to experimental data of pH, texture (Tx), raw batter stability (RBS) and water holding capacity (WHC) were determined using the Pure Error and Statistical Significance (p) and are presented in Table 3. Since the purpose of fractional design was to determine the intensity of effects and their significance, not

the validation of models, no need to evaluate the effects of residual error.

The coefficient p is the level of significance of the independent variable on the response under study. It was chosen as the confidence interval value of 95%. Thus, we can say that for p values less than 5%, the variable is considered statistically significant.

Table 3 Estimated effect, pure error and degree of statistical significance (p) for the parameters pH, Tx, RBS and WHC

Variables	Effect Estimated	Pure error	t(2)	Significance Statistical (p)
a) pH				
Ms (%)	−0.047	0.010	−4.398	0.048
Tsp (%)	−0.037	0.010	−3.472	0.073
F (%)	−0.057	0.010	−5.323	0.033
Cg (%)	−0.017	0.010	−1.620	0.246
b) Tx				
Ms (%)	−43.972	14.899	−2.951	0.098
Tsp (%)	−54.052	14.899	−3.627	0.068
F (%)	−57.652	14.899	−3.869	0.060
Cg (%)	−58.072	14.899	−3.897	0.059
c) RBS				
Ms (%)	0.777	1.939	0.400	0.727
Tsp (%)	0.937	1.939	0.483	0.676
F (%)	0.112	1.939	0.058	0.959
Cg (%)	0.992	1.939	0.511	0.659
d) WHC				
Ms (%)	−0.015	0.004	−3.674	0.066
Tsp (%)	−0.005	0.004	−1.224	0.345
F (%)	−0.025	0.004	−6.123	0.025
Cg (%)	0.035	0.004	8.573	0.013

Ms manioc starch; Tsp texturized soybean protein; F hydrogenated soy fat; Cg carrageenan; Tx texture; RBS raw batter stability; WHC water holding capacity

Table 4 Values of the levels of the variables studied in the complete factorial design and the responses of pH, Tx, RBS and WHC experimentally obtained in the processing of sausages

Testing	Ms (%)	F (%)	Cg (%)	pH	Tx (g)	RBS (%)	WHC
1	1 (−1)	1 (−1)	0.25 (−1)	7.13	388.75	88.16	0.46
2	2 (+1)	1 (−1)	0.25 (−1)	7.11	317.66	89.11	0.35
3	1 (−1)	2 (+1)	0.25 (−1)	7.43	214.50	85.94	0.64
4	2 (+1)	2 (+1)	0.25 (−1)	7.69	231.00	87.14	0.27
5	1 (−1)	1 (−1)	0.75 (+1)	7.06	327.30	89.38	0.38
6	2 (+1)	1 (−1)	0.75 (+1)	7.04	315.50	88.58	0.32
7	1 (−1)	2 (+1)	0.75 (+1)	7.31	342.00	88.33	0.52
8	2 (+1)	2 (+1)	0.75 (+1)	7.27	302.50	91.01	0.32
9	0.66 (− α)	1.5 (0)	0.5 (0)	7.23	188.00	86.12	0.61
10	2.34 (+ α)	1.5 (0)	0.5 (0)	7.27	356.00	89.23	0.20
11	1.5 (0)	0.66 (− α)	0.5 (0)	7.32	194.50	88.96	0.77
12	1.5 (0)	2.34 (+ α)	0.5 (0)	7.26	172.00	87.97	0.63
13	1.5 (0)	1.5 (0)	0.08 (− α)	7.29	222.00	89.15	0.42
14	1.5 (0)	1.5 (0)	0.92 (+ α)	7.23	277.50	89.87	0.44
15	1.5 (0)	1.5 (0)	0.5 (0)	7.29	377.50	91.14	0.47
16	1.5 (0)	1.5 (0)	0.5 (0)	7.38	373.50	91.04	0.47
17	1.5 (0)	1.5 (0)	0.5 (0)	7.20	387.50	90.46	0.48

Ms manioc starch; F hydroge-nated soy fat; Cg carrageenan; Tx texture; RBS raw batter stability; WHC water holding capacity
n=3

It appears in Table 3a that the factors were significant with 95% confidence for the concentration of manioc starch and vegetable fat content, since the concentrations of texturized soybean protein and carrageenan were not significant. The biggest effect was the vegetable fat variable, which has negative effects.

For the sausage texture parameter (Table 3b) it appears that, the independent variables showed no significant effects to $p>0.05$, and therefore have no effect on the texture response. In this case, the concentration of manioc starch, textured protein, vegetable fat and carrageenan had no significant effect to the sausage texture.

Notes on Table 3c independent variables have no significant effects $p>0.05$. The carrageenan exerted a major influence on the raw batter stability (RBS), followed by texturized soybean protein concentration and manioc starch.

Evaluation of the effects of independent variables on the responses of WHC is presented in Table 3d. It appears that the carrageenan was the variable that showed the greatest effect on the response of WHC followed by hydrogenated soy fat with 95% confidence. The manioc starch and texturized soybean protein showed no significant effect on the response.

Based on the results, texturized soybean protein, was not significant for any of the responses studied, and then removed from the complete factorial design applied to study the best formulation of piramutaba sausages.

Complete factorial design 2³

Table 4 presents the values of pH, texture (Tx), raw batter stability (RBS), and water holding capacity (WHC), obtained experimentally in the preparation of sausages with fat

Table 5 Results of the analysis of variance for pH, Tx, RBS and WHC

Source	SQ	GL	MQ	F _{calculated}	F _{tabulated} (p ≤ 0.1)	R ²
pH						
Regression	0.118	1	0.118	7.07	3.07	0.31
Waste	0.251	15	0.016			
Lack of fit	0.235	13	0.018	2.23	9.41	
Pure error	0.016	2	0.008			
Total	0.369	16				
Tx						
Regression	48671.57	3	16223.856	6.85	2.56	0.61
Waste	30773.23	13	2367.171			
Lack of fit	30669.23	11	2788.111	53.61	9.40	
Pure error	104	2	52			
Total	79444.8	16				
RBS (%)						
Regression	35.687	8	4.46	9.91	2.58	0.9
Waste	3.608	8	0.45			
Lack of fit	3.339	6	0.55	4.23	9.32	
Pure error	0.269	2	0.13			
Total	39.295	16				
WHC						
Regression	0.242	3	0.08	10	2.56	0.69
Waste	0.105	13	0.008			
Lack of fit	0.105	11	0.009	272.72	9.4	
Pure error	0.000	2	0.000			
Total	0.347	16				

SQ quadratic sum; GL degree of freedom; MQ mean square root; Tx texture; RBS raw batter stability; WHC water holding capacity

Fig. 1 Response surface and level curve for RBS, relating hydrogenated soy fat and manioc starch

substitutes, following the complete experimental design worksheet 2³.

The pH values found were higher than those found in literature and in the raw material (waste), this is mainly due to the presence of polyphosphates added to the residue (Yang et al. 2001). According Hughes et al. (1997) the pH value affects the emulsion because of its effect on the myofibrillar proteins that reaches its maximum emulsifying capacity at pH near neutral. Moreover, the greater the amount of protein solubilized better the emulsion, because the greater amount of emulsifying agent, making very important its meat extraction.

According to Eleya and Turgeon (2000) pH is not important factor for the gelation of κ -carrageenan, but at pH below 4.0 the gelation may not occur due to acid hydrolysis. The proteins with amphoteric character precipitate with carrageenan if the solution pH is below the isoelectric point. In solutions that have pH values above the isoelectric point strong interaction occurs between proteins and carrageenan (Eleya and Turgeon 2000; Hua et al. 2003).

Yoon et al. (2006) when studying the influence of various levels of fat in sausages, they found lower pH values in the formulations where the amount of fat was lower.

According to Grinberg and Tolstoguzov (1997) and Syrbe et al. (1998), the pH effects on the behavior of protein-

polysaccharide mixture differ depending on the nature of the polysaccharide (neutral, carboxylated or sulfated).

Andrs et al. (2006) when using agar hydrocolloids and xanthan gum, in chicken sausage obtained a reduction in hardness by increased concentrations of hydrocolloids in the formulations. Pedroso and Demiate (2008) found similar results by using carrageenan as a fat substitute in baked ham of turkey.

Pietrasik (2003) found that samples of meat products, prepared with kappa-carrageenan had a substantial increase in hardness; in addition, the author states that carrageenan interacts with proteins to improve yield and texture of poultry meat products, especially when high water levels are used.

Viana et al. (2003) argue that the partial withdrawal of the fat on the binding properties of meat products, has been the subject of discussion by some authors, because some studies claim that when the fat reduction is accompanied by an increase in the proportion of water, a fact found in this study, these products exhibit low binding properties (water holding capacity and raw batter stability).

The high water retention capacity found in piramutaba sausage, differs from the results found by Cengiz and Gokoglu (2008), by using fat replacer of frankfurter-type sausages.

Fig. 2 Response surface and level curve for RBS, relating carrageenan and manioc starch

Fig. 3 Response surface and level curve for RBS, relating carrageenan and hydrogenated soy fat

Lyons et al. (1999) also showed an increased ability to retain water when prepared sausages with low fat adding whey protein, carrageenan and manioc starch in the substitution of fat.

Another study found similar to the results of this study was to Zayas and Lin (1989) which showed a high capacity to retain water after the addition of 3% protein of defatted corn germ to the mass of raw sausage. Viana et al. (2003) stated that in relation to the binding properties of meat emulsions, the effects of fat replacing are contradictory.

Eliminating factors not significant, there was a significant regression for 90% confidence ($p \leq 0.1$), the F test in analysis of variance. Table 5 presents the results of variance analysis for pH, Tx, RBS, WHC and the values calculated and tabulated F.

It was found in Table 5 that the models obtained for pH, texture and water holding capacity (WHC), however, showed low coefficients of determination (0.31, 0.61, 0.69), indicating that the variation percentage explained by the regression were 31, 61 and 69%, respectively, which can be confirmed by the values of F calculated. Therefore, there is not a good adjustment of the experimental data to models and cannot be relieved its response surfaces.

The proposed model for the raw batter stability (RBS) was significant and predictive as shows the analysis of variance. The coefficient of determination (R^2) was 0.90, indicating that the model explained 90% of the variation of the experimental data. The F value calculated for the regression was greater than the tabulated F indicating that the model is predictive. Thus, the proposed model to represent the raw batter stability (RBS), depending on manioc starch, hydrogenated soy fat and carrageenan is shown below (Eq. 2):

$$\text{RBS\%} = 90.87 + 0.67F - 1.12F^2 - 0.32F - 0.84F^2 + 0.59C - 0.47C^2 + 0.46FG + 0.69GC \quad (2)$$

Where: *Ms*: Manioc starch; *F*: hydrogenated soy fat; *Cg*: Carrageenan.

The response surfaces generated by the proposed model for raw batter stability (RBS) considering the average points

of manioc starch, hydrogenated soy fat and carrageenan in the sausages emulsion are illustrated in Figs. 1 and 3.

It appears in Figs. 1, 2 and 3 that the greater raw batter stability (RBS) of the sausages can be obtained in concentration ranges: carrageenan of 0.51 to 0.8%, manioc starch of 1.45 to 1.85% and hydrogenated soy fat of 1.2 to 1.7%. It was chosen an optimum condition for the formulation of piramutaba sausage (final product): carrageenan (0.51%), manioc starch (1.45%) and hydrogenated soy fat (1.2%).

According to Brasil (2000) is permitted the addition of at most 2% of manioc starch in sausage, if the product contains levels above those permitted by Regulation (RIISPOA), it shall submit the label the amount of ingredient added.

The interaction between proteins and polysaccharides has a direct influence on the macroscopic properties of foods, such as fluidity, stability, texture and mouth feel (Eleya and Turgeon 2000; Hua et al. 2003). According to Shand and Smith (1997), the functional properties of meat systems also suffer the influence of protein interactions with other components such as fat, protein and water.

Table 6 Microbiological analysis of piramutaba sausage

Analysis	Sausage	Legislation ^a
<i>Coliforms</i> at 45 °C (MPN/g) ^b	7	Max 10 ³
<i>Salmonella</i> (25 g)	Absence	Absence
<i>Staphylococci coagulase</i> ⁺ (UFC/g) ^c	<1 × 10 ²	Max 3 × 10 ³
<i>Clostridium</i> sulfite reducer	Negative	Max 5 × 10 ²
Molds and yeasts (CFU/g)	<1.5 × 10 ²	–
<i>Psychrophiles</i> (CFU/g)	<3 × 10 ²	–

^a Brazilian law (Brasil 2001)

^b MPN most probable number

^c CFU colony forming unit

n=3

Final formulation of piramutaba sausage

The conditions chosen for concentration of Ms, F and Cs were used for the final formulation of piramutaba sausage consisted of: surimi 70.0%, ice 20.0%, manioc starch 1.5%, texturized soybean protein 1.45%, hydrogenated soy fat 1.2%, carrageenan 0.51%, curing salt 0.2%, refined salt 1.0%, condiments for sausage 1.5%, sodium tripolyphosphate 0.3%, sodium erythorbate (antioxidant) 0.3%, monosodium glutamate 0.5%, cochineal powder 0.02%. Sausages with this formulation were evaluated microbiologically.

Microbiological characterization

The results of samples microbiological analysis of piramutaba sausages are presented in Table 6.

Resolution of RDC N.12, January 2, 2001, National Sanitary Surveillance Agency - ANVISA (Brasil 2001), establishes the maximum tolerance allowed in fecal coliform for sausages 10^3 MPN/g, so in the sample of sausage, values were below the limit determined by law and, therefore, in accordance with established standards.

It was not detected *Salmonella* in samples of sausage and can be considered suitable for consumption. This bacterium does not exist originally in the fish, being introduced during handling or by contact with contaminated water and its presence is a major problem for public health because of its pathogenic character (Al-Bulushi et al. 2011). According to Spricigo et al. (2008) the presence of *Salmonella* in food indicates inadequacy of the product for consumption and the bacteria of this genus are primarily responsible for food-borne outbreak.

The results found for coagulase staphylococci is within the standards of current law that allows a maximum of 10^3 CFU/g for products made from refrigerated fish, including built-in fish (Brasil 2001).

The Ministry of Health (Brasil 2001) allows a maximum of 3×10^3 CFU/g for clostridium sulphite reducer embedded in meat, so not detected in the built-in piramutaba indicating good conditions for preparing and storing the product. The curing salts used in sausages are nitrate and nitrite that generate inhibitory substances of microorganisms especially against *Clostridium botulinum* (Honikel 2008).

Although the legislation does not establish parameters for the standard count in plate of psychrotrophic, the microbiological standards established by the National Norms and Standards for Foods of the Ministry of Health for raw fish, fresh, chilled, frozen, including fillets and breaded states that the standard score (maximum) of mesophilic allowed in plate is 10^6 CFU/g. Based on the results of the sausage samples analyzed showed values less than that set by legislation. Oliveira Filho et al. (2010) also obtained similar results to psicrotrophic bacteria in sausages elaborated with

Nile tilapia indicating good conditions and shelf life of 40 days when stored at 0 ± 0.3 °C.

There are no standards in the legislation for molds and yeast, but the result of this analysis showed that the sausage had satisfactory score in the case of spoilage microorganisms.

Conclusion

On the binding properties of emulsions, the water retention capacity and raw batter stability had effects contradictory to those found in the literature.

Piramutaba sausages can be prepared using as fat substitutes carrageenan, manioc starch and hydrogenated soy fat in concentrations of 0.51, 1.45 and 1.2% respectively.

Samples of piramutaba sausage were within the microbiological standards established by law, considered suitable for consumption.

Acknowledgments This study received financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brasil).

References

- Al-Bulushi IM, Kasapis S, Dykes GA, Al-Waili H, Guizani N, Al-Oufi H (2011) Effects of frozen storage on the characteristics of a developed and commercial fish sausage. J Food Sci Technol. doi:10.1007/s13197-011-0441-x
- Ambrosiadis J, Varelzis KP, Georgakis SA (1996) Physical, chemical and sensory characteristics of cooked meat emulsion style products containing vegetable oils. Int J Food Sci Tech 31:189–194
- Andrs S, Zaritzky N, Califano A (2006) The effect of whey protein concentrates and hydrocolloids on the texture and colour characteristics of chicken sausages. Int J Food Sci Tech 41:954–961
- Auh J, Kim Y, Cornillon P, Yoon J, Yoon S, Park K (2003) Cryoprotection of protein by highly concentrate branched oligosaccharides. Int Food Sci Tech 38:553–563
- Barreto LM, Beiro LH (1999) Influncia do amido e carragena nas propriedades texturais de surimi de tilpia (*Oreochromis* sp). Cienc Tecnol Alimen 19:183–188. doi:10.1590/S0101-20611999000200005
- Bhattacharyya D, Sinhamahapatra M, Biswas S (2007) Preparation of sausages from spent duck-an acceptability study. Int J Food Sci Technol 42:24–29
- Brasil (2000) Ministrio da Agricultura, Regulamentos tcnicos de identidade e qualidade de carne mecanicamente separada, de mortadela, de lingia e de salsicha. Available at: <http://extranet.agricultura.gov.br/sislegis-consulta/servlet/VisualizarAnexo?id=1638> [Accessed Nov 15 2011]
- Brasil (2001) Ministrio da Sade, Agncia Nacional de Vigilncia Sanitria-ANVISA. Resoluo RDC n 12 de 02 de janeiro de 2001. In: Regulamento Tcnico sobre os padres microbiolgicos para alimentos. Dirio Oficial, Braslia, DF. Available at: http://www.anvisa.gov.br/legis/resol/12_01rdc.html [Accessed Mar 12 2010]
- Cengiz E, Gokoglu N (2008) Effects of fat reduction and fat replacer addition on some quality characteristics of frankfurter-type sausages. Int J Food Sci Technol 43(3):366–372

- Chen HH (2000) Effect of non-muscle protein on the thermogelation of horse mackerel surimi and the resultant cooking tolerance of kamaboko. *Fisheries Sci* 66:783–788
- Eleya MO, Turgeon SL (2000) The effects of pH on the rheology of β -lactoglobulin/ κ -carrageena mixed gels. *Food Hydrocolloids* 14:245–251
- Fabré NN, Barthem RB (2005) O manejo da pesca dos grandes bagres migradores: piramutaba e dourada no eixo Solimões-Amazonas. Manaus: Ibama, ProVárzea p 114. Available at: <http://www.pirada.org/download/anais/bagres.pdf> [Accessed Nov 17 2011]
- Gonçalves AA, Nogueira WM, Lourenço LH (2009) Aproveitamento do descarte do processamento da piramutaba (*Brachyplatystoma vaillantii*) e do camarão-rosa (*Farfantepenaeus subtilis*) na produção de salsicha sabor camarão. *Bol Inst Pesca* 35(4):623–635. Available at: ftp://ftp.sp.gov.br/ftpcesca/35_4_623-635.pdf [Accessed 25 Nov 2011]
- Grinberg VY, Tolstoguzov VB (1997) Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids* 11:145–158
- Hermansson AM (1980) Effect of processing conditions on microstructure and functional properties of comminuted meat batters. *Proceedings of European Meeting of Meat Research Workers*, Colorado Springs, CO, p 134
- Honikel KO (2008) The use and control of nitrate and nitrite for the processing of meat products. *Meat Sci* 78(1):68–76
- Hu Y, Xia W, Ge C (2008) Characterization of fermented silver carp sausages inoculated with mixed starter culture. *LWT - Food Sci Technol* 41(4):730–738
- Hua Y, Cui SW, Wang Q (2003) Gelling property of soy protein-gum mixtures. *Food Hydrocolloids* 17(6):889–894
- Hughes E, Cofrades S, Troy DJ (1997) Effects of fat level, oat fibre and carrageenan on frankfurters formulated with 5, 12 and 30% fat. *Meat Sci* 45:273–281
- Keeton JT (1994) Low-fat meat products technological problems with processing. *Meat Sci* 36(2):261–276
- Lyons PH, Kerry JF, Morrissey PA, Buckley DJ (1999) The influence of added whey protein/carrageena gels and tapioca starch on the textural properties of low fat pork sausages. *Meat Sci* 51:43–52
- Mehta NK, Elavarasan K, Manjunatha Reddy A, Shamasundar BA (2011) Effect of ice storage on the functional properties of proteins from a few species of fresh water fish (*Indian major carps*) with special emphasis on gel forming ability. *J Food Sci Technol*. doi:10.1007/s13197-011-0558-y
- MPA (Ministério da Pesca e Aquicultura) Boletim Estatístico da Pesca e Aquicultura Brasil (2009) Available at: <http://www.mpa.gov.br/mpa/seap/Jonathan/mpa3/docs/anuário%20da%20pesca%20completo2.pdf> [Accessed 20 Nov 2011]
- Oliveira Filho PR, Netto FM, Ramos KK, Trindade MA, Viegas EM (2010) Elaboration of sausage using minced fish of Nile tilapia filleting waste. *Braz Arch Biol Technol* 53(6):1383–1391. doi:10.1590/S1516-89132010000600015
- Olivo R, Shimokomaki M (2006) Emulsões Cárneas. In: Shimokomaki M, Olivo R, Terra NN, Franco BD (eds), *Atualidades em Ciência e Tecnologia de Carnes*. Varela, São Paulo, pp 123–133
- Park JW, Morrissey MT (2000) Manufacturing of surimi from light muscle fish. In: Park JW (ed), *Surimi and Surimi Seafood*. Marcel Dekker, New York, pp 23–37
- Pedroso RA, Demiate IM (2008) Avaliação da influência de amido e carragena nas características físico-químicas e sensoriais de presunto cozido de peru. *Cienc Tecnol Alimen* 28:24–31. doi:10.1590/S0101-20612008000100005
- Pietrasik Z (2003) Binding and textural proprieties of bee gels processed with κ -carrageena, egg albumin and microbial transglutaminase. *Meat Sci* 63:317–324
- Sachindra NM, Mahendrakar NS (2010) Stability of carotenoids recovered from shrimp waste and their use as colorant in fish sausage. *J Food Sci Technol* 47(1):77–83
- Santiago PA, Piñeiro C, González MJ (2004) Quality loss related to rancidity development during frozen storage of horse mackerel (*Trachurus trachurus*). *JAOCs* 81(7):671–678
- Shand CA, Smith S (1997) Enzymatic release of phosphate from model substrates and P compounds in soil solution from a peaty podzol. *Biol Fertil Soils* 24:183–187
- Siddaiah D, Reddy GVS, Raju CV, Chandrasekhar TC (2001) Changes in lipids, protein and kamaboko forming ability of silver carp (*Hypophthalmichthys molitrix*) minced during frozen storage. *Food Res Int* 34(1):47–53
- Spricigo DA, Matsumoto SR, Espindola ML, Ferraz AS (2008) Prevalência, quantificação e resistência a antimicrobianos de sorovares de *Salmonella* isolados de lingüiça frescal suína. *Cienc Tecnol Aliment* 28(4):779–785. doi:10.1590/S0101-20612008000400003, 784
- Su YK, Bowers JA, Zayas JF (2000) Physical characteristics and microstructure of reduced-fat frankfurters as affected by salt and emulsified fats stabilized with non meat proteins. *J Food Sci Technol* 65:128–132
- Syrbe A, Bauer WJ, Klostermeyer H (1998) Polymer science concepts in dairy systems—an overview of milk protein and food hydrocolloid interaction. *Int Dairy J* 8:179–193
- Tornberg E (2005) Effect of heat on meat proteins—implications on structure and quality of meat products. *Meat Sci* 70:493–508
- Vanderzant C, Splittstoesser DF (1992) *Compendium of methods for microbiological examination of foods*, 3rd edn. American Public Health Association, Washington, p 914
- Viana FR, Silva VDM, Bizzotto CS, Laboissière LHS, Drumond MFB, Oliveira AL, Silvestre MPC (2003) Globina e plasma bovinos, como substitutos de gordura em patê de presunto: efeito da incorporação sobre a composição química, textura e características sensoriais. *Alim Nutr* 14(1):77–85. Available at: <http://serv-bib.fcfar.unesp.br/seer/index.php/alimentos/article/view/841/721> [Accessed 25 Jan 2012]
- Yang A, Keeton JT, Beilken SL, Trout GR (2001) Evaluation of some binders and fat substitutes in low-fat frankfurters. *J Food Sci* 66(7):1039–1046
- Yoon SD, Chough SH, Park HR (2006) Effect of additives with different functional groups on the physical properties of starch/PVA blend films. *J Appl Polym Sci* 100:3733–3740. doi:10.1002/app.23303
- Zayas JF, Lin CS (1989) Effect of the pretreatment of corn germ protein on the quality characteristics of frankfurters. *J Food Sci* 54:1452–1456
- Zhang J, Liu Z, Hu Y, Fang Z, Chen J, Wu D, Xingqian Y (2011) Effect of sucrose on the generation of free amino acids and biogenic amines in Chinese traditional dry-cured fish during processing and storage. *J Food Sci Technol* 48(1):69–75