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FULL PAPER

Improvement of protein content and effect on technological properties of wheat bread with the addition by cobia (*Rachycentron canadum*)

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Abstract

The aim of this work was to improve the protein content of the wheat bread and to study the technological properties, incorporating Cobia (*Rachycentron canadum*) minced proteins (CMP). The influence of CMP on the characteristics of wheat bread, such as texture, color, specific volume (SV), protein and lipids content were evaluated. Wheat flour was replaced by CMP at different levels of substitution, and hydrogenated vegetable fat was added in different levels according to 2^2 central composite rotational design (CCRD) as shown: CMP: from 2.00 to 12.00%; hydrogenated vegetable fat: from 0.00 to 3.00%. As control was elaborated wheat bread without the addition of CMP. Analysis of the breadcrumb color with the addition of CMP at 3.50% and vegetable fat up to 1.50% showed the highest lightness. The highest SV values were obtained with an amount of 0.44% of vegetable fat in the formulation. However, the increase in the addition of CMP resulted in a reduction of SV. The addition of CMP and vegetable fat results in an antagonistic effect on breadcrumb firmness; the higher the vegetable fat and the lower the CMP contents resulted in higher softness. The CMP incorporation in bread caused a protein increase in the range of 40 to 70% compared to wheat bread without CMP.

1. Introduction

The availability of nutrients and the supply of proteins to humanity is an important and vital question in the world. It is a problem in developing countries where the population increased is not accompanied by the supply of high quality protein (Moscatto *et al.,* 2004). The health of several people could be enhanced by increasing protein nutritional value of wheat flour bread (Cercel *et al.,* 2016). This fact occurs because, according to some authors (Gómez *et al.,* 2008; Turfani *et al.,* 2017), wheat bread is a popular food worldwide. It is a source of calories and of complex carbohydrates (Cercel *et al.,* 2016; Turfani *et al.,* 2017). An alternative of wheat bread protein enrichment is the incorporation of vegetable or animal protein from different sources as lentil, carob, faba bean sourdough, fish (Cercel *et al.,* 2016; Coda *et al.,* 2017; Turfani *et al.,* 2017).

According to Brazilian law bread is the product obtained from wheat flour and/or other flours, added with other ingredients, resulting from fermentation and cooking process, that may contain other ingredients, as long as they don't change the characteristics of the products (Brazil, 2005). However, the wheat flour bread contains low levels of essential amino acids such as lysine and threonine (Cercel *et al.,* 2016; Pallarés *et al.,* 2007; Turfani *et al.,* 2017). In several countries, due to the significant role of a diet based on wheat bread, it has become a habit to enrich flour with nutrients that are limited or absent in this flour, according to the law of each country (Centenaro *et al.,* 2007; Keiko *et al.,* 2011).

The essential amino acids concentration in the diet is one of the most important factors in the nutritional value of a food protein. Proteins derived from animal sources, such as fish, are considered nutritionally superior to that of plant origin, they contain a better balance of essential amino acids (Kristinsson and Rasco, 2000). Fish is an excellent source of protein, due to the essential amino acids and protein content between 15 and 25% (Zhou *et al.,* 2004). Among warm-water marine fishes, cobia (*R. canadum*) is one of the best aquaculture candidate species in the world (Benetti *et al.,* 2010). Cobia is a marine fish native to Brazil, neritic and with migratory behavior and is widely distributed in tropical and subtropical waters of all oceans, except the Central and Eastern Pacific. Cobia does have favorable characteristics to be qualified such as high rates of growth, good feed conversion, and adaptation to environmental captivity, low mortality, high market value as well as excellent meat quality (Benetti *et al.,* 2010; Coriolano and Coelho, 2012).

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Several studies about the influence of the addition of protein from different sources on wheat bread physical-chemical, technological and functional properties have been reported in the last years (Cercel *et al.,* 2016; Swieca *et al.,* 2017; Coda *et al.,* 2017; Turfani *et al.,* 2017). The characteristics of wheat bread as physical attributes of texture, color and specific volume (SV) are among the most important characteristics taken into account by consumers. The mechanical properties of bread are often associated with the perception of freshness and elasticity and influence the consumption decision (Angioloni and Collar, 2009). In this context, the aim of this work was to evaluate bread added by cobia minced protein to evaluate the protein increase and effect on the technological properties.

2. Materials and methods

2.1 Material

The fish species used in this study was cobia (*R. canadum*), provided by Marine Aquaculture Station (MAE) of the Federal University of Rio Grande (FURG). Commercial wheat flour type I, according to the Brazilian flour classification was provided by Moinho Galópolis S.A from the city of Rio Grande, Southern Brazil. This wheat flour showed a 76.29%, 9.80%, 9.40%, 1.40% and 0.14% content for carbohydrates, protein, moisture, lipids, and ash, respectively. The most of ingredients used in the production of the wheat bread as hydrogenated vegetable fat, sodium chloride, sucrose and dried yeast, were purchased at the local market. All other chemicals were of analytical grade.

2.2 Preparation of cobia minced protein

Cobia minced protein (CMP) was performed according to Centenaro *et al.* (2007), with modifications. Cobia was washed in chlorinated water at 5 ppm at 4°C followed by beheaded and gutted. Then, fishes were processed in a meat-bone separator (High Tech, HT250, Brazil) that discarded skin and bones resulting in mechanically separated meat (MSM). The CMP from MSM was obtained by washing process in distilled water (at ratio 1:3; w/v; minced/water) for 5 min at constant stirrer and filtered through a layer of nylon cloth. This washing process was repeated three times. The CMP was centrifuged in a hydro-extractor (Anko,YL-15, Taiwan), during this process the cobia minced protein was separated from excess of water. The CMP obtained was lyophilized (Liotop, L108, Brazil) at -55°C and 50 μHg for 48 h, ground in a knife-mill (Tecnal, TE-633, Piracicaba, Brazil), sieved through a No. 42 mesh (0.35 mm) and stored at - 18°C until use.

2.3 Wheat bread making

The ingredients used for bread making are reported in Table 1. Flour, sodium chloride, sucrose and ascorbic acid, were initially homogenized using a planetary mixer (Kitchen-Aid, 300 Stand Mixer, Brazil) followed by addition of vegetable fat and water at maximum speed for 10 min until the gluten had completely developed. Wheat flour and hydrogenated vegetable fat were added at different levels as follow: CMP: 2.00, 3.45, 7.00, 10.55 and 12.00%; hydrogenated vegetable fat: 0.00, 0.44, 1.50, 2.56 and 3.00%. All values are expressed in relation to wheat flour.

Bread dough was cut into pieces weighing 175 g, which were subsequently molded into rounded spherical shaped forms with its own cylinder. Then, they are placed in metallic molds with the following dimensions: 13.3 x 5.5 cm^2 of the smaller base and 15.5 x 7.4 cm^2 of larger base and height of 4.5 cm. The fermentation of shaped doughs was carried out in a stove (Qumis, Q 317M, Brazil) at 30°C for 90 min with controlled relative humidity (80%). The doughs were then baked in an electric oven (Malta, 45LT, Brazil) at 200°C for 20 min. After 1 h of baking at room temperature, the bread were sliced with an electric knife, (Moulinex, 51302, France) for further analysis.

2.4 Technological and physical-chemical characteristics of bread

2.4.1 Crumb firmness

Table 1. Wheat bread formulation

Ingredients	Bread with cobia minced proteins		Control bread	
	%	Dough (g)	%	Dough (g)
Wheat Flour	88 to 98	294 to 264	100	300
Dry Cobia minced	2 to 12	6 to 36	-	-
Sugar (sucrose)	5	15	5	15
Sodium chloride (NaCl)	2	6	2	6
Vegetable fat	0 to 3	0 to 9	2	6
Ascorbic acid	0.009	0.027	0.009	0.027
Dry yeast	1.5	4.5	3	9
Water	60	180	60	180

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The crumb firmness of the wheat bread was performed in triplicate in the texturometer ($TAXT_{Plus}$, Stable Micro Systems, England) according to the method described by AACC (2000) (74-09.01). The cylindrical probe of 36 mm in diameter was utilized at following conditions: speed of 1.0 mm/s for the pre-test; speed of 1.7 mm/s for the test; speed of 10.0 mm/s for the post-test; 40% compression and 5-g trigger force. The crumb firmness values were expressed as g-f (gram-force).

2.4.2 Crumb color

The color of the crumb, CMP, and wheat flour were determined in a colorimeter (Konica Minolta, CR-400, Japan) using the D_{65} standard illuminant according to International Commission on Illumination (CIE, 1976) in triplicate. Results were expressed in the L*, a*, b* color space corresponding to lightness, chromaticity on a green (-) to red (+) axis, and chromaticity on a blue (-) to yellow (+) axis, respectively. The wheat flour showed L*, a*, b values of 94.00, - 0.21 and 9.71 and the cobia minced protein showed 83.36, 0.72 and 11.78 values, respectively.

2.4.3 Specific volume

Specific volume (SV) was measured from the ratio between the apparent volume (mL/g) in triplicate by displacement of millet seeds and its weight according to AACC (2000) based on method number 72-10.

2.4.4 Protein and lipids content

The protein and lipid contents of the CMP and wheat bread were determined in triplicate according to the methods of the AOAC (2000).

2.5 Experimental design and statistical analysis

The 2^2 central composite rotational design (CCRD) was performed with four factorial points, three central points, and four axial points, totaling 11 assays, according to Box and Wilson (1951) as shown in Table 2. The CCRD was used to analyze the effects of the following parameters: CMP (2-12 %) and hydrogenated vegetable fat (0-3 %) content on the dependent variables: physical-chemical and technological properties of wheat bread. The interaction between the variables was evaluated using the software Statistica (Version 5.0, by StatSoft, Inc., Tulsa, USA) using a confidence interval of 95%.

3. Results and discussion

3.1 Bread characteristics

Table 2 presents the results of technological and physical-chemical characteristics of bread as responses in the experimental design by 2^2 central composite rotational design. Data were fitted as functions of the dependent variables. Equations (1)–(3) represent the coded models for Lightness (L*), specific volume (SV) and firmness and are shown in Table 3. According to the variance analysis (ANOVA), the models represented by these equations were significant at a 95% level of significance ($p < 0.05$). Thus, the contour surfaces were generated for the statistically significant model (Box; Wilson, 1951) that are shown in Figures 1a, 1b, and 1c. However, the a* and b* were not significantly correlated ($p > 0.05$)

3.1.1 Crumb color

Color is one of the most important parameters in the appearance of baked products that contributes to the consumer's preference (Fernandes and Salas-Mellado, 2017). Table 2 presents the color values, dependent variables that were determined for wheat bread in which the wheat flour was replaced by CMP at different levels of substitution. In most of the experiments, it is observed that crumb lightness (L*) decreases as the replacement of CMP and addition of vegetable fat increased, as shown in Figure 1a. The wheat bread of F4 experiment formulated with 10.55% of CMP and 2.56% of vegetable fat, showed the lower lightness (L* = 68.26) in the crumbs than the other bread elaborated. This can be explained by the addition of high levels of CMP (10.55%). This effect was expected because of the darker color of CMP (L*= 83.36, a*= 0.72, b*=11.78) than that wheat flour (L*=94.00, a*= -0.21, b*= 9.71), respectively. The highest lightness was obtained in bread of the F1 experiment (L* = 73.38) elaborated with 3.45% of CMP and 0.44% vegetable fat. The CMP substitution at levels lower than 3.45%, and vegetable fat lower than that 1.50% results in the attainment of higher lightness in the bread crumbs, respectively. According to Esteller *et al.* (2005), the increase in the internal temperature to 98° C in the baking process results in technological and physical changes as starch gelatinization and changes in gluten proteins. This contributes to a forming of a golden and fine crust formed due to sugars caramelization and Maillard reaction.

According to Rodriguez Furlán *et al.* (2015), the lower values of L* (+) indicate darker coloring, due to interactions between the ingredients activated by heating. This fact is more pronounced in formulations with a greater presence of sugars. In this study all experiments showed that crumb of bread was all about a value of a* = - 4.00, indicating that the green component was pronounced as shown in Table 2.

In this study, a yellowness (b*+) coloration was obtained with the addition of CMP. This result is in

Table 2. Matrix of the experimental design and values of color, specific volume and firmness of breads. * Percentages in relation to the quantity of flour (g/100 g)

Experiment	Independent variables		Crumb color			Specific Volume (mL/g)	Firmness (g)
	Minced Coded variable X_1	Fat Coded variable X_2	L*	a*	b*		
F1	-1 (3.45)	-1 (0.44)	73.38 ± 1.10	-4.51 ± 0.15	21.49 ± 0.51	2.72 ± 0.07	1412.21 ± 93.57
F2	+ 1 (10.55)	-1 (0.44)	70.63 ± 1.23	-4.11 ± 0.12	21.83 ± 0.44	2.28 ± 0.05	1973.02 ±116.56
F3	-1 (3.45)	+1 (2.56)	71.64 ± 0.79	-4.59 ± 0.15	20.87 ± 0.53	3.09 ± 0.05	925.98 ± 98.96
F4	+ 1 (10.55)	+ 1 (2.56)	68.26 ± 1.34	-4.31 ± 0.14	22.09 ± 0.48	2.37 ± 0.02	1919.79 ± 52.75
F5	-1.41 (2.00)	0 (1.50)	72.12 ± 1.38	-4.68 ± 0.17	20.18 ± 0.69	3.05 ± 0.05	796.31 ± 47.60
F6	1.41 (12.00)	0 (1.50)	69.02 ± 1.19	-4.30 ± 0.21	21.98 ± 0.40	2.07 ± 0.01	2020.70 ± 89.95
F7	0 (7.00)	-1.41 (0)	72.45 ± 0.78	-4.20 ± 0.10	21.49 ± 0.51	2.31 ± 0.02	1941.79 ±101.48
F8	0 (7.00)	1.41 (3.00)	70.50 ± 1.01	-4.56 ± 0.11	22.09 ± 0.42	2.39 ± 0.05	1411.13 ±120.59
F9	0 (7.00)	0 (1.50)	70.86 ± 1.02	-4.44 ± 0.10	21.89 ± 0.29	2.53 ± 0.02	1367.93 ± 29.19
F10	0 (7.00)	0 (1.50)	69.87 ± 1.16	-4.56 ± 0.16	21.93 ± 0.27	2.47 ± 0.04	1248.62 ± 33.45
F11	0 (7.00)	0 (1.50)	70.67 ± 0.60	-4.33 ± 0.09	21.84 ± 0.40	2.52 ± 0.03	1487.24 ± 39.61

Table 3. Contour surface regression models for the parameters lightness (L*), specific volume (SV) and Firmness

Parameters	Equations	R^2	F	F calculated/ F tabulated
Lightness (L*)	$L = 70.85 – 1.32 X_1 – 0.86 X_2$	0.88	29	6.50
Specific volume	$VE = 2.53 – 0.32 X_1: + 0.07 X_2$	0.86	24.29	5.45
Firmness	$Firmness = 1549.04 + 411.35 X_1 - 161,44 X_2$	0.82	17.90	4.01

X1: Cobia minced protein; X2: Hydrogenated vegetable fat; R2: coefficient of determination ($p < 0.05$); F: test F ($p < 0.05$).

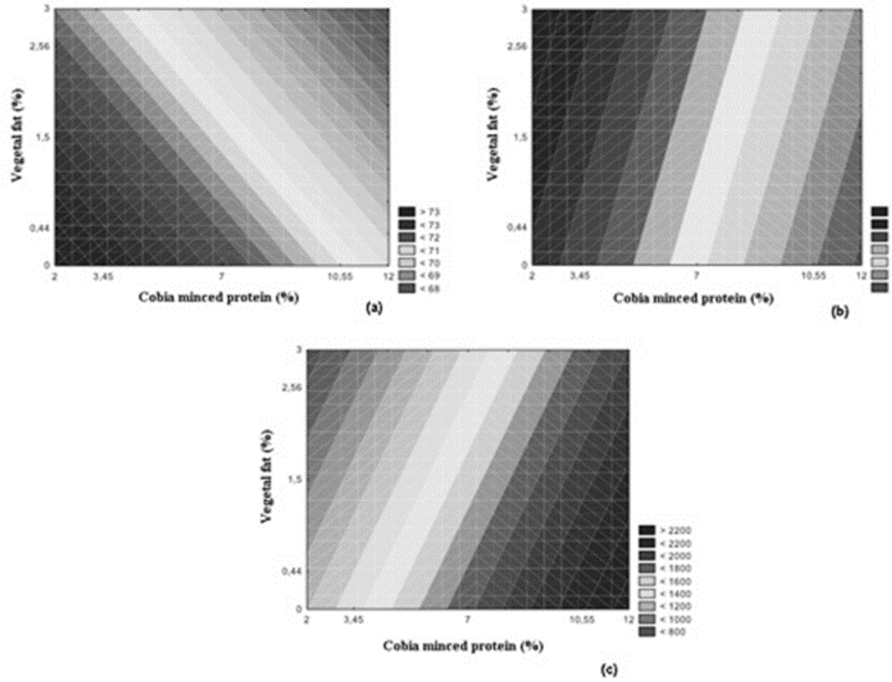

Figure 1. Contour curves for brightness (L*) (1a), specific volume (1b) and firmness (1c) as a function of Cobia minced protein – CMP and vegetable fat

agreement with the previous study by Ziobro *et al.* (2013) that found that breadcrumb with the addition of pea and soy protein displayed highest values of b* (yellowness). According to Esteller and Lannes (2005), the high b* values were presented in samples with strong yellowish or golden color (chestnut color, typical of baked products), that appears in bread rich in protein.

3.1.2 Specific Volume

Specific volume (SV) is one of the most important parameters of bakery products that are influenced by type, quality and amount of ingredients used (Felisberto *et al.,* 2015; Fernandes and Salas-Mellado, 2017). Table 2 presents the SV of wheat bread where wheat flour was replaced by CMP at different levels of substitution. The results showed that highest values of SV were obtained with addition from 0.44% to 3.00% of vegetable fat as shown in Figure 1b. According to Fernandes and Salas-Mellado (2017), vegetable fat possesses a property of promoting aeration of the dough, influencing the specific volume. The increase of CMP levels in wheat bread resulted in a decrease of SV as shown in Figure 1b. The SV values obtained were similar to those found by Centenaro *et al*. (2007), who studied the effect of dry washed minced from *Bluewing searobin* on technological quality of wheat bread. They observed a decrease in SV values when minced was used at 50% level, leading to low values of SV (2.29 mL/g). Cercel *et al.* (2016) also reported that wheat bread with fish protein concentrated induced a decrease in SV compared to control bread. Esteller and Lannes (2005) reported that bread with low specific volume showed a disagreeable appearance to the consumer. According to Ortolan and Steel (2017), the ability of the proteins present in the flour to expand and retain the gas formed inside the dough of the bread occurs due to the ability of the gluten present in the wheat protein. These authors reported that animal proteins do not possess this characteristic.

3.1.3 Crumb firmness

The firmness of the bread is related to the force applied to cause deformation or rupture of the sample. The maximum force measured for bakery products is dependent on the formulation (flour quality, quantity of sugars, fats, emulsifiers, enzymes, addition of gluten and flour improvers), dough moisture and conservation (manufacturing time and product packaging) (El-Dash, 1978). According to Angioloni and Collar (2009), the crumb hardness is used as a measure of the aging of bread, therefore softer bread provide the feeling of "new". The effect of the addition of CMP on crumb firmness is presented in the values shown in Table 2.

The lowest wheat bread crumb firmness (796.31 g-f) was obtained in experiment F5, with the low addition of vegetable fat (2.00%), and an greater crumb firmness (2020.70 g-f) was found in the wheat bread with higher CMP addition (F6 experiment, 12.00%). It is observed that the addition of CMP and vegetable fat resulted in an antagonistic effect on crumb firmness of the bread, as shown in Figure 1c, where higher vegetable fat content and lower CMP showed the higher softness of the breadcrumb. These results demonstrated that the texture of the wheat bread was negatively affected by adding CMP in the formulation, possibly due to alteration in the formation of the gluten network. However, Ziobro *et al.* (2013) verified that the incorporation of other protein types as lupine protein showed a decrease in bread hardness. Comparably to the results of this study, the incorporation of faba bean flour, for protein enrichment of wheat bread, by Coda *et al*. (2017) caused a higher hardness compared to wheat bread without faba bean flour.

3.1.4 Protein and fat content

Figure 2 represents the protein and lipids as ingredients used in the preparation of bread. The F5, F6 and F9 experiments showed good technological properties as SV and firmness. The addition of 2.00 and 12.00% of CMP on formulations of experiments F5, F9, and F6 experiments resulted in an increase in total protein content of 43.87, 67.35 and 93.41% and a decrease in lipids content of 10.22 and 3.95% for F5 and F9 compared to control wheat bread.

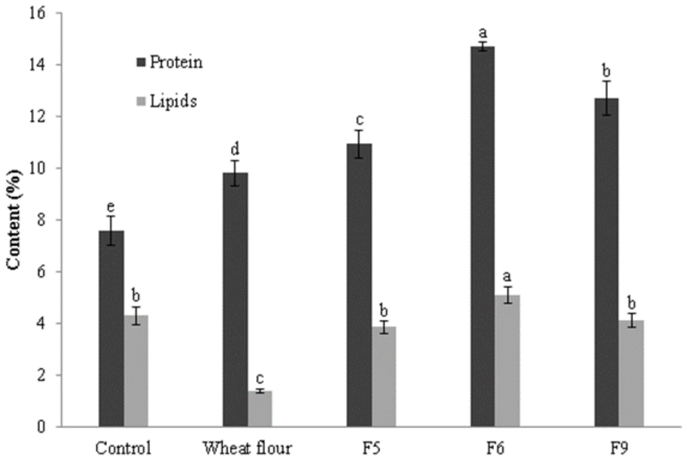

Figure 2. Protein and lipids content of Control bread, Wheat flour and F5 (2.00% of CMP and 1.50% of vegetable fat), F9 (7.00% of CMP and 1.50% of vegetable fat) and F6 (12.00% of CMP and 1.50% of vegetable fat) experiments.

Centenaro *et al.* (2007) elaborate bread enriched with minced meat of Bluewing searobin and verified that control bread showed a 11.8% of total protein (on wet basis),15.5 % and 17.1 % for bread added with 3 and 5% of dry minced, respectively. However, those authors did not verify a significant difference in lipids content with the increase in minced meat of Bluewing searobin in the bread. Adeleke and Odedeji (2010) evaluated the quality of wheat bread elaborated by fortification of wheat flour with tilapia fish protein flour in varying proportions (5, 10, 15 and 20%) by wheat flour. Those authors verified that protein content increased with the increasing quantity of tilapia protein added. Furthermore, the increase in protein content of bread, did not alter the sensory characteristics such as taste, flavor, crust and crumb color, and nor the overall acceptance when the product was compared with control bread. Bolarinwa *et al.* (2017) elaborated bread fortified with moringa (*Moringa oleifera*) seed powder at varying proportions (0–20%) and verified that wheat bread sample fortified with 20% moringa powder had the highest (13.5%) protein content while the un-fortified bread had the least

(8.6%) protein, respectively. According to these authors the higher amount in total protein content of the fortified bread could be due to the high total protein content of moringa seed used as fortificant. The increment in the protein content of the breads in the present study could be due to the high protein content of cobia minced protein used as substituted of wheat flour.

4. Conclusion

This study showed that when wheat flour was replaced by Cobia minced protein at different levels it influenced on technological and physical-chemical characteristics as lightness, specific volume and crumb firmness of wheat breads. The increase in the addition of cobia minced protein resulted in decrease of lightness and specific volume and an increase in crumb firmness. Compared to control wheat bread, the addition of cobia minced protein showed a bread protein increase in the range 40 to 70%. The addition of vegetable fats to bread conferred a contrary effect to that of the minced fish, increasing the softness of the bread crumb.

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