Fish filleting residues for enrichment of wheat bread: chemical and sensory characteristics

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Abstract The fish processing industry generates a large amount of materials discarded as residue. It is known that fish and its residue are a source of essential nutrients. Conversely, bread is a food accessible to different social classes but is deficient in protein, minerals, and fatty acids. Thus, this study aimed to develop bread products based on the addition of flour from Red-tailed Brycon (*Brycon cephalus*) processing residue and then evaluate the chemical and sensory qualities of the products. In relation to a standard bread formulation without fish flour, the addition of fish flour to bread formulations resulted in products with not only higher contents of protein, essential fatty acids, and minerals, especially calcium and phosphorus, but also lower contents of carbohydrates. The breads with fish flour received sensory acceptance better than or as good as that of a bread formulation without fish flour. Hence, the addition of fish processing residue to breads is a way to provide essential nutrients to the population through a well-accepted, accessible, and low-cost product.

Keywords Bread · Fish residue · Proximate composition · Sensory analysis · Fatty acids

Introduction

The fish processing industry generates a large amount of materials discarded as residue during the operations of filleting or slicing. These residues, which are rich in organic

and inorganic materials, can pollute natural resources (air, water, and soil) and serve as nutrients for the development of pathogens harmful to human health if released into the environment without prior treatment. For the production of fish to become environmentally viable, it is necessary to find alternatives that fully exploit the fish, preventing large amount of residue (Carvalho et al. 2006).

The use of these residual materials in food can mitigate the environmental problem and has a number of advantages due to the high nutritional value of low-cost fish residue (Souza et al. 2004). Fish and its residue are an easily digestible source of high biological value protein, essential fatty acids, and minerals (especially calcium and phosphorus) as well as vitamins A, D, and B complex, all of which make for a product of high nutritional value (Henderson and Tocher 1987; Kristinsson and Rasco 2000).

Fish also represents a major dietary source of polyunsaturated fatty acids, especially the omega-3 series, such as eicosapentaenoic acid (EPA-C20:5W3) and docosahexaenoic acid (DHA-C22:6W-3) (Kotb et al. 1991; Ramírez et al. 2001). These have been associated with numerous health benefits for humans, such as reductions in levels of cholesterol and triglycerides or prevention of cardiovascular disease, hypertension, diabetes, cancer, and multiple sclerosis (Herold and Kinsella 1986; Sargent 1997).

Despite the nutritional importance of fish, some people have low levels of consumption, probably because fish is a highly perishable food susceptible to microbiological contamination, short shelf life, and high cost, among other factors. An interesting alternative to increased consumption of fish per se would be a diversification of the processing line through the development of new products (Bordignon et al. 2010).

Currently, both consumers and food companies are increasingly concerned with healthy eating. In this context, the enrichment of foods with nutrients has been an accepted practice

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by food industries since the mid-twentieth century and aims to enhance the nutritional value by preventing or correcting deficiencies in one or more nutrients (Reilly 1996). Bread is a food accessible to different social classes due to its low price and market availability, but it is deficient in protein, minerals, and essential fatty acids. Hence, bread has been the subject of many studies on food enrichment (Ilyas et al. 1996; Conto et al. 2012).

Previous studies showed that fish processing residue can be utilized in the preparation of flour for human consumption (Benites and Souza-Soares 2010). Thus, the present study aimed to evaluate the influence of the partial replacement of wheat flour by fish residue flour on the chemical composition and sensory quality of sliced bread.

Experimental

Preparation of fish residue flour

The fish residue used to produce the flour was obtained from the filleting of Red-tailed Brycon (*Brycon cephalus*). Head, bones, viscera, and skin were used to obtain the fish residue flour. These residues were initially washed and subjected to steaming for 25 min. Subsequently, the material was ground in a stainless steel mill and placed in pans to be dried in a forced air circulation incubator (model Q314M, Quimis) at 60 °C for about 7 h. The material resulting from the drying was ground in a bladed blender (Pic-Liq, Arno) and standardized in a Tyler 20 sieve (0.85 mm) to finally obtain the fish residue flour. The flour was vacuum-packed in polyethylene bags and stored in a refrigerator (4 °C) for 2 days until the preparation of bread.

Preparation of the breads

Wheat flour (Antonella), refined iodized salt (Cisne), sugar (União), dough enhancer (Zea), fresh compressed yeast, and

fish flour (from Red-tailed Brycon residue) were used for the production of bread according to Table 1.

The direct dough system was used in the preparation of bread (Lorenz and Kulp 1991). A trough (Supreme SR 30) was initially used on low speed to mix the dry ingredients (flour, salt, sugar, and dough enhancer) and then on high speed until the development of gluten was complete. The fish flour was added during the initial phase of the mixing of the dry ingredients. Then, the yeast was added and homogenized in the trough until the development of gluten was complete. This required the addition of a quantity of water, which varied according to the amount of fish flour and the need to achieve the standard dough consistency. The standard bread formulation (without fish flour) was similarly obtained using only wheat flour (F1, Table 1).

The dough was cut into 300 g portions that were molded and shaped by hand. The dough portions were placed in metal forms (15.5×7.5×4.5 cm) and taken to ferment for 40 min at room temperature (25 °C). Finally, the loaves of bread were baked at 165 °C for 30 min in an electric furnace (Diplomata, Fischer) and cooled at room temperature for 1 h. The chemical and sensory analyses were performed on the first day of storage.

Fifteen units of bread for each formulation were produced and used in the analysis of proximate composition, mineral content, fatty acid profile and sensory analysis. For sensory analysis, each bread was sliced into ten pieces.

Proximate composition

The breads were analyzed for chemical composition according to the AOAC official method (Hernandez et al. 2005). Lipids were determined by the Soxhlet method. Total protein was determined by the Kjeldahl method using content of N×6.25 for fish and content of N×5.7 for bread. Ash and moisture were determined by gravimetric methods at 600 °C (muffle) and 105 °C (oven), respectively.

Minerals contents

Fe, Zn, and Ca contents were analyzed after destroying the organic matter of the samples by dry ashing for 12 h with a final temperature of 500 °C. The ash was dissolved with 3 mL of concentrated HNO₃ acid on a hot plate, and the volume was made up to 25 mL with distilled deionized water. The minerals were measured by atomic absorption spectrophotometry, using a Varian SpectraAA 110 spectrophotometer with an air-acetylene flame and monoelemental hollow cathode lamp for each element (Hernandez et al. 2005). Phosphorus was measured by the colorimetric molybdenum ammonium vanadate method (AOAC 1995), using a Shimadzu UV 1800 spectrophotometer.

Table 1 Formulations used for the preparation of bread with replacement of wheat flour by fish flour at different concentrations

	F1 (%)	F2	F3	F4	F5
Fish flour	0	4.2	8.4	12.6	16.8
Wheat flour	84	79.8	75.6	71.4	67.2
Yeast	4	4	4	4	4
Sugar	5	5	5	5	5
Salt	2	2	2	2	2
Dough enhancer	5	5	5	5	5

F1 standard bread with no fish flour, F2 bread with 5 % fish flour, F3 bread with 10 % fish flour, F4 bread with 15 % fish flour, F5 bread with 20 % fish flour

Fatty acids profile

Fat from bread samples was extracted by Soxhlet into diethyl ether. The extract was transferred to a glass vial and then evaporated to dryness at 40 °C under nitrogen stream. 2.0 mL BF_3 reagent (7 % in methanol) and 1.0 mL toluene were added. The mixture was heated at 100 °C for 45 min, shaking every 10 min. The mixture was cooled to room temperature and 5.0 mL H_2O , 1.0 mL hexane and 1.0 g Na_2SO_4 were added. The mixture was stirred for 1 min. The layers were separated and then the top layer (containing fatty acid methyl esters – FAMES) was carefully transferred to another vial containing 1.0 g Na_2SO_4 . FAMES were determined in a Shimadzu GC2010 gas chromatograph equipped with a FAME column and a flame ionization detector (FID) using helium as the carrier gas. The oven temperature was ramped at 3 °C min^{-1} from 100 °C (hold 4 min) to 240 °C (hold 15 min). The fatty acids were identified by comparison of retention times using mixed FAME standards (AOAC 1995).

Sensory analysis

The bread formulations (Table 1) were evaluated by 100 untrained adult consumers. The attributes evaluated were appearance, taste, texture, global aspect, and purchase intention.

The acceptance test was performed using a 9-point hedonic scale (9 = like extremely, 1 = dislike extremely) and the purchase intention test using a 5-point scale (5 = definitely would buy the product, 1 = certainly would not buy the product).

The tests were conducted in an enclosed cabin with white illumination. Samples were placed on a white workbench and coded with three random digits.

Statistical analysis

The proximate and mineral composition data of the breads were compared by Tukey's honestly significant difference test at the 95 % confidence level. Mean groups were indicated by the same letter.

Principal Component Analysis (PCA) was applied to the fatty acid composition data in order to evaluate the differences and similarities among the formulations and to highlight the typical composition. The dataset, consisting of 5 formulations $\times$ 17 variables (identified fatty acids), was autoscaled and subjected to PCA.

Sensory data were statistically analyzed by means of a three-way internal preference mapping (Nunes et al. 2011), which allows for an evaluation of the acceptability of the samples according to the hedonic scores of consumers and considering the acceptance information of several attributes simultaneously. The three-way preference map was obtained by Parallel Factor Analysis (PARAFAC) (Bro 1997), from a

three-dimensional dataset consisting of 5 samples $\times$ 80 consumers $\times$ 6 attributes.

The calculations were performed using Matlab version 7.5.

Results and discussion

Chemical composition

The addition of fish flour in partial substitution for wheat flour resulted in changes in the chemical compositions of breads. Carbohydrate levels were significantly reduced with the addition of fish flour (Table 2). The reduction occurred probably due to a low content of carbohydrates (and high protein) in fish residues (Benites and Souza-Soares 2010). The standard formulation with no fish flour (F1) had the highest carbohydrate content (562 g kg^{-1}). The carbohydrate content was reduced with the replacement by larger proportions of fish flour. The formulation with the highest level of fish flour (F5) was characterized by the lowest level of carbohydrates (454 g kg^{-1}) in its composition. Despite this decrease in carbohydrate content, all of the formulations are good sources of carbohydrates, because these provide 10–19 % of the daily value (DV) per reference amount customarily consumed (RACC) of 100 g according to the FDA (FDA 2008). Studies indicate that an excessive intake of carbohydrates is associated with a higher prevalence of metabolic syndrome, so the food industry has encouraged researchers to develop foods with reduced levels of carbohydrates.

Bread is a food deficient in proteins of high biological value, but the enrichment of formulations with fish flour increased the protein content (Table 2). The breads with added fish flour of 10 % (F3), 15 % (F4), and 20 % (F5) can be considered excellent sources of protein, because each contains more than 20 % of the DV (50 g day^{-1}) per RACC (100 g) according to the FDA recommendations (FDA 2008). Importantly, the proteins derived from animal sources, like fish, are considered nutritionally superior to those from vegetal sources because the former contain a better balance of essential amino acids (Diniz and Martin 1996).

Table 2 Proximate compositions of the bread formulations enriched with fish flour

	F1 (g kg^{-1})	F2	F3	F4	F5
Carbohydrates	562 $\pm$ 11 ^a	533 $\pm$ 7 ^b	508 $\pm$ 1 ^b	482 $\pm$ 4 ^c	454 $\pm$ 1 ^d
Proteins	84 $\pm$ 6 ^a	102 $\pm$ 3 ^b	121 $\pm$ 1 ^c	131 $\pm$ 1 ^{cd}	142 $\pm$ 3 ^d
Lipids	21 $\pm$ 1 ^a	29 $\pm$ 1 ^b	36 $\pm$ 2 ^{bc}	42 $\pm$ 3 ^c	53 $\pm$ 1 ^d
Minerals	16 $\pm$ 1 ^a	18 $\pm$ 1 ^a	22 $\pm$ 1 ^b	24 $\pm$ 1 ^{bc}	26 $\pm$ 1 ^c

Different superscripts in the same row indicate that means were significantly different ($P < 0.05$). Mean of three replications

It is known that fish is a source of lipids, especially fatty acids essential to humans (Henderson and Tocher 1987; Ackman 2005). Hence, the addition of fish flour caused a significant increase in the total amounts of fat in the breads (Table 2). However, all of the formulations can be classified as low fat because each has less than 5 g of fat per RACC (100 g) according to the FDA (FDA 2008).

The profile of lipid composition (Table 3) reveals a greater diversity of fatty acids from an increased replacement of wheat flour by fish flour.

A more detailed exploration of the fatty acid profile was performed by PCA (Fig. 1). The first principal component (PC1), which explained the most variance (61.00 %), distributed the formulations in order of increasing replacement of wheat flour by fish flour.

The standard formulation (F1) is characterized by a higher content of *cis*-linoleic (C18:2n6c), γ -linolenic (C18:3n6), myristic (C14:0), and lauric (C12:0) acids compared to the other formulations. Insofar as the formulations shift rightward along the PC1 axis, an increase in the content of oleic (C18:1n9c), palmitoleic (C16:1), *cis*-eicosenoic (C20:1n9), and palmitic (C16:0) acids was observed with the increase in fish flour content. The formulation F5 was highly correlated to behenic (C22:0), eicosadienoic (C20:2), lignoceric (C24:0), pentadecanoic (C15:0), and eicosatrienoic (C20:3n3) acids. According to Table 2 these glycerides were identified only in this formulation, although in small amounts relative to other fatty acids detected. In general, the addition of fish flour contributed an increase to the content of unsaturated fatty acids, such as *cis*-eicosenoic, palmitoleic, and mainly *cis*-

Fig. 1 PCA biplot of scores and loadings for fatty acid profile of the bread formulations enriched with fish flour

oleic. Conversely, the content of saturated fatty acids, such as myristic, lauric, and elaidic (C18:1n9t), was slightly decreased.

Fatty acids, mono- and polyunsaturated, are considered very important for some aspects of human health. The *cis* forms of these acids have been associated with various beneficial health effects, such as decreased total and low-density lipoprotein (LDL), anticarcinogenic properties, and decreased cardiovascular risk (Kris-Etherton and Innis 2007). Thus, the dietary recommendations emphasize a reduced intake of saturated and *trans* fatty acids, with an increased intake of polyunsaturated.

Regarding minerals, fish is considered a valuable source of calcium and phosphorus in particular, and it provides moderate amounts of other minerals (Centenaro et al. 2007). According to Table 4, the standard bread with no fish flour (F1) showed small amounts of Ca (2.5×10^{-2} g kg⁻¹). However, by adding the fish flour, a progressive and significant increase in Ca was observed. The formulations with 10 %

Table 3 Fatty acids profile of the bread formulations enriched with fish flour

	F1	F2	F3	F4	F5
C10:0	–	–	–	0.2	0.1
C12:0	3.3	2.0	0.8	2.0	1.4
C14:0	2.1	1.6	1.3	1.6	1.5
C15:0	–	–	–	–	0.1
C16:0	20.4	20.5	22.8	23.5	24.3
C16:1	1.0	1.2	1.7	1.8	1.9
C17:0	–	–	–	0.4	0.3
C18:0	11.7	12.3	12.6	10.0	9.6
C18:1n9t	3.3	4.0	2.3	2.4	1.7
C18:1n9c	26.5	35.0	36.2	37.3	40.0
C18:2n6c	28.9	19.9	14.7	17.2	20.6
C18:3n6	2.8	1.6	1.1	1.4	1.5
C20:1n9	–	0.6	0.6	0.8	1.1
C20:2	–	–	–	–	0.2
C22:0	–	–	–	–	0.2
C20:3n3	–	–	–	–	0.3
C24:0	–	–	–	–	0.1

Table 4 Mineral compositions of the bread formulations enriched with fish flour

	Minerals ($\times 10^{-2}$ g kg ⁻¹)				
	F1	F2	F3	F4	F5
P	105 $\pm$ 7 ^a	145 $\pm$ 7 ^b	207 $\pm$ 11 ^c	225 $\pm$ 7 ^c	305 $\pm$ 7 ^d
Ca	2.5 $\pm$ 3.5 ^a	14 $\pm$ 5 ^a	112 $\pm$ 18 ^b	190 $\pm$ 14 ^c	350 $\pm$ 14 ^d
Zn	1.7 $\pm$ 0.1 ^a	1.8 $\pm$ 0.1 ^a	2.2 $\pm$ 0.4 ^a	2.2 $\pm$ 0.3 ^a	2.8 $\pm$ 0.1 ^b
Fe	5.1 $\pm$ 0.2 ^a	5.4 $\pm$ 0.2 ^a	5.7 $\pm$ 0.1 ^a	5.8 $\pm$ 0.2 ^a	6.4 $\pm$ 0.6 ^b

Different superscripts in the same row indicate that means were significantly different ($P < 0.05$). Mean of three replications

(F3), 15 % (F4), and 20 % (F5) additions of the fish flour can be considered fortified, enriched, or extra sources of Ca according to the FDA (FDA 2008), because these provide more than 10 % of the DV (1 g day^{-1}). In addition, formulation F5 can be considered an excellent source of calcium, since it provides 36 % of the DV per RACC.

The addition of fish flour to bread also provided a significant increase in the levels of P. According to the results, formulation F2 can be considered a good source of P, because it provides 15 % of the DV (1 g day^{-1}) per RACC (100 g). The formulations F3, F4, and F5 provide more than 20 % of the DV and therefore are considered rich or excellent sources of P according to the FDA (FDA 2008).

The Zn content of formulation F1 was not statistically different from those of formulations F2, F3, and F4, differing only from that of formulation F5. The recommended DV of Zn for adults and children 4 or more years of age corresponds to 15 mg according to the FDA (FDA 2008). However, ingestion of one RACC (100 g) of formulation F5 meets 39.07 % of the DV, which makes this formulation rich in Zn. All of the formulations with fish flour can also be considered rich or excellent sources of Fe, since these formulations provide more than 20 % of the DV (18 mg per RACC of 100 g).

Skrbic and Filipcev (2007) reported that a 300 g intake of white bread provides about 10 % of the DV for calcium and 30 % of that for Fe. However, as discussed above, the fish breads developed in this study can be considered of high nutritional value because these provide a mineral content superior to that of similar common breads.

Sensory analysis

The bread formulations underwent sensory evaluation by untrained adult consumers for appearance, taste, texture, global aspect, and color on a 9-point hedonic scale and for purchase intention on a 5-point scale. The sensory acceptance data were analyzed by means of a three-way internal preference map. The method is based on data decomposition by PARAFAC, which is a method for decomposition of higher order data and can be considered a generalization of the PCA to multidimensional data (Bro 1997). Preference maps obtained by PARAFAC provide graphical interpretations of sensory data from consumers simultaneously considering the various sensory attributes under evaluation (Nunes et al. 2011).

The best PARAFAC model was obtained with three factors and presented an explained variance of 48.8 % and core diagnostic consistency of 85.4 % (Nunes et al. 2011; Bro 1997). Figure 2 presents the three-way internal preference map for the sensory data of the bread formulations. Considering all the sensory attributes evaluated, a greater preference for formulations F2 and F3 was observed, since

Fig. 2 Three-way internal preference map for appearance (app), texture (tex), purchase intention (pin), color (col), taste (tas), and global aspect (gas) of the bread formulations enriched with fish flour. Consumers are represented by vectors

there is a greater number of consumers (vectors) in the direction of these samples. The greater preference for these formulations can be attributed mainly to the higher acceptance scores for texture, global aspect, taste, and color. The purchase intent and appearance scored well in the cases of formulations F1, F2, and F3. However, the standard formulation (F1), despite having received good scores for purchase intent and appearance, was not preferred in general by consumers. The formulations F4 and F5, despite not being among the most preferred, for global aspect received mean scores between 6 and 7, which corresponds to “like slightly” or “like moderately” on the hedonic scale. Thus, these formulations can also be regarded as well accepted by consumers.

Conclusion

In relation to a standard bread formulation without fish flour, the addition of Red-tailed Brycon processing residue flour to sliced bread formulations resulted in products with higher content of protein, essential fatty acids, and minerals (especially calcium and phosphorus) in addition to lower content of carbohydrates.

For appearance, taste, texture, global aspect, color, and purchase intention, the bread formulations with fish flour received sensory acceptance better than or as good as that of a standard bread formulation without fish flour.

Hence, the addition of fish processing residue to breads is a way to provide essential nutrients to the population through a well-accepted, accessible, and low-cost product.

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