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Production of protein bars with Nile tilapia protein concentrate

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Abstract

Commercial protein bars such as whey protein bars (New Millen® protein complex premium) are consumed worldwide and are usually made with whey as a protein source. Fish protein concentrate (FPC) is an alternative protein of animal origin with a different amino acid profile and fatty acid composition and can be produced from mechanically separated meat (MSM) of Nile tilapia (*Oreochromis niloticus*). This study investigated FPC for its nutritional characteristics and microbiological quality for the manufacture of protein bars. The FPC-enriched bars presented a potential profile of essential fatty acids for human consumption. The sensory evaluation showed good acceptance scores, with no differences from the control (commercial product) and high purchase intention. The inclusion of 5.3% of FPC in the protein bars with different flavors may be a promising alternative to produce a high nutritional value product for human consumption, besides favoring the sustainability of the fish supply chain.

Keywords: waste from continental fishing industry; mechanically separated meat; sustainability.

Practical Application: Fish protein concentrate (FPC) is an alternative source for the composition of protein bars, meeting the nutritional and microbiological requirements and with acceptability by consumers.

1 INTRODUCTION

Consumer profiles have changed in recent decades, with an increased demand for healthy protein-rich foods produced in a sustainable way. The supply and consumption of protein derived from fish have also increased intensively in recent decades (FAO, 2022). In this sense, fishing, which used to be the main fish source, went into decline and the supply expanded mainly due to the cultivation of aquatic organisms (FAO, 2022). In turn, aquaculture has been growing due to greater food security by providing animal protein of good nutritional quality (FAO, 2022) and product traceability.

Nile tilapia is one of the main fish species produced in Brazil, due to its peculiar characteristics, including omnivorous eating habits, easy management, resistance to temperature and diseases, and white and mild-tasting meat (Peixe BR, 2022). Although tilapia farming has great local socioeconomic importance, the amount of solid waste generated in the filleting process is of great concern. Solid waste consists of the head, spine, skin, fins, and viscera, which can cause damage to the environment when not correctly disposed of (Feltes et al, 2009; Pessatti, 2001). The use of solid waste from the filleting process can be an effective alternative to minimize the environmental impacts, besides providing a new production line, generating jobs, and increasing the economic development of the fish supply chain (Arruda, 2004; Seibel & Soares, 2003).

The tilapia carcass residue with bones and remaining meat is mainly used for the production of fishmeal. However, the technological development of the industry has provided modern techniques and equipment to separate parts of the muscle residue from the fish bones, giving rise to mechanically separated meat (MSM). This fish co-product along with processing techniques allows the production of fish protein concentrate (FPC), which has emerged as an alternative for the use of fish waste (Pereda et al, 2005; Sebben et al., 2000). The process of obtaining FPC is based on fish delipidation and protein extraction, with subsequent deodorization (Kirschnik, 2007).

Fish protein-enriched products have high nutritional value and are considered sources of proteins, carbohydrates, unsaturated fatty acids, and vitamins, which can promote an adequate and healthy diet for the population with an exhausting routine that many times makes adequate nutrition impossible. Thus, FPC provides innovative and practical possibilities for a quick, easy, and affordable diet (Pessatti, 2001), with an emphasis on protein bars.

The bars are classified into three types: those containing a proportional combination of carbohydrates, protein, and fat, those containing high amounts of carbohydrates and less fat, and those containing considerable amounts of protein. A though high-protein bars (or simply protein bars) were originally developed for athletes and competitors, there has been great

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consumer demand in recent years, often as sports supplements (Loveday et al., 2009).

Protein intake is extremely important for athletes because the predominance of protein synthesis is needed to increase muscle mass and improve the performance of the athlete (Bezerra & Macêdo, 2013; Haraguchi et al., 2006). Whey protein is obtained during the cheese-making process and is the most commonly used protein source in the manufacture of protein bars, often in blends with soy protein isolate. From a chemical point of view, whey proteins have significant amounts of calcium and high nutritional value, containing a high content of essential amino acids, especially branched-chain amino acids (BCAA), including leucine, isoleucine, and valine, with approximately 25.1%. It also has alanine (15–25%), tryptophan (6%), cysteine, lysine, threonine, and cystine, among others. In turn, it contains lower amounts of aromatic amino acids (phenylalanine and tyrosine) that exert important nutritional properties (Haraguchi et al., 2006; Sgarbieri, 2004).

In this sense, the protein concentrate from MSM of Nile tilapia was used to produce protein bars with different flavors and compared with protein bars made with a commercial formulation based on whey protein (New Millen® protein complex premium). All FPC-enriched bars and the whey protein-based bar (New Millen®) were characterized for nutritional and microbiological characteristics, fatty acids profile, Aw, pH, and sensory evaluation.

2 MATERIALS AND METHODS

This project was approved by the Ethics Committee of the Universidade Estadual de Maringá (UEM) under CAEE Registration number 71048517.2.0000.0104 (Resolution 196/96 of the National Health Council).

2.1 Manufacture of Nile tilapia protein concentrate

The FPC was made from Nile tilapia carcass (spine with meat remaining from the filleting process) without the head, which was obtained after filleting at Smart Fish Company in the city of Rolandia (PR). The carcasses were frozen and transported to the Fish Technology Laboratory at Fazenda Experimental de Iguatemi (FEI), belonging to the UEM.

To produce the MSM, the fins were removed from the carcasses and washed. The carcasses were passed through an HT 250 pulping machine to obtain MSM. Then, the batter was subjected to four washing cycles. For the first and second cycles, the batter was placed in a cloth bag and 200% water at 5°C in relation to the weight of MSM was added, with agitation for 5 min and removal of excess water. For the third washing cycle, 200% water at 5°C and 0.03% phosphoric acid were used, with agitation for 15 min and removal of excess water. For the fourth washing cycle, the procedure of the first cycle was repeated, followed by centrifugation of the MSM for 13 min to remove excess water. After all washing cycles, the material was placed in a recipient with 100% water at 100°C, 0.1 mg/kg peroxitane, and 0.5 mg/kg antioxidant butyl-hydroxy-toluene (BHT) and subjected to cooking for 30 min. The cooked MSM was pressed in a hydraulic press, with a capacity of 10 tons, and part of the water and fat was removed. The remaining protein mass was placed in a drying oven with air circulation at 90°C for 24 h to produce the FPC. After drying, the product was ground in a knife mill (Willye – TE-650 model), vacuum packed, and stored in a freezer (-18°C) until the manufacture of the protein bars.

2.2 Preparation of FPC-enriched protein bars

Four protein bar formulations were made (Table 1), with the addition of 5.3% of commercial protein complex (New

Table 1. Protein bar formulations of different flavors.

	Bars			
	Commercial protein complex Banana	FPC with flavor		
		Banana	Orange/apple	Peanut
Dry ingredients (%)				
Sweet potato	26.50	26.50	26.50	26.50
Oat flakes	17.65	17.65	17.65	17.65
Cocoa powder	4.50	4.50	4.50	4.50
Ginger	0.40	0.40	0.40	0.40
Culinary sweetener	1.30	1.30	1.30	1.30
Cinnamon powder	0.40	0.40	0.40	0.40
Clove powder	0.20	0.20	0.20	0.20
Banana	17.75	17.75	–	–
Orange/apple	–	–	17.75	–
Peanut	–	–	–	17.75
Protein complex	5.30	–	–	–
FPC	–	5.30	5.30	5.30
Wet ingredients (%)				
Skim milk	17.70	17.70	17.70	17.70
Egg white	7.00	7.00	7.00	7.00
Coconut oil	1.30	1.30	1.30	1.30
Topping (chocolate)	–	–	–	–

Millen®) vanilla flavor for the control and the addition of 5.3% of FPC for the other treatments with different flavors (banana, orange/apple mix, and peanut). The fruits were macerated and homogenized, and the peanuts were ground before the addition to the formulations.

The ingredients were weighed in stainless steel bowls according to each formulation (Table 1, Figure 1A) and homogenized. The mixture was placed in a mold and heated in an oven at 180°C for 30 min. Afterward, the bars were cut approximately 2 cm wide and 4 cm long and covered with melted dark chocolate (Figure 1B). The bars were individually packed and stored under refrigeration (5°C) for the microbiological analysis and sensory evaluation and under freezing (-14°C) for the determination of the proximate composition and fatty acids profile.

2.3 Microbiological characterization, water activity, pH, and instrumental color measurements of the FPC-enriched bars

The microbiological analyses of FPC and the protein bars were performed in the Food Microbiology and Microscopy laboratory of the Clinical Analysis Department at the UEM. The most probable number (MPN) of coliforms at 35 and 45°C, coagulase-positive staphylococci counts in colony-forming units (CFU)/g, and the presence of *Salmonella* spp. in 25 g of the sample were determined according to APHA (2001).

The microbiological protocol followed the standards recommended by Resolution RDC 12 of January 2, 2001, of the National Health Surveillance Agency (Brasil, 2001).

Figure 1. (A) Preparation of protein bars (ingredients and fish protein concentrate (arrow)) and (B) protein bar ready for consumption.

The pH measurement was performed in the homogenized sample (10 g) with distilled water (1:10 sample/water) using a pH meter electrode (DM 22, Digimed, São Paulo, Brazil) for 5 min.

The Aw of the samples was determined using the apparatus Aw Sprint – Novasina TH-500.

To measure the instrumental color of the protein bars, a portable colorimeter (model MiniScan EZ, Hunter Lab brand) was used, with a D65 light source, 10º observation angle, and 30 mm measuring cell opening, using the L*, a*, and b* scale of the CIELAB system (Hunter, 1975). The parameters L*, which defines the luminosity (L* = 0 black and L*=100 white), a* (red-green component), and b* (yellow-blue component) (OIV, 2006) were determined. Four readings were taken for each sample, totaling five samples per treatment.

2.4 Proximate composition of FPC and FPC-enriched bars

The proximate composition was determined in the laboratory of Food Chemistry of the Group of Studies and Management in Aquaculture (GEMAq) of the Universidade EStadual do Oeste do Paraná (UNIOESTE). The moisture, lipids, protein, and ash contents of the FPC and the protein bars were determined according to the methodologies of AOAC (2005), in triplicate, and the carbohydrate levels were calculated by difference. The total energy value was obtained by the sum of protein, lipid, and carbohydrate values multiplied by factors 4, 9, and 4, respectively (Souci et al., 2000).

2.5 Fatty acid profile of FPC and FPC-enriched bars

The fatty acid profile was determined according to Hartman and Lago (1973). For that, approximately 100 mg of lipids from each treatment was transmethylated, using a solution of ammonium chloride and sulfuric acid in methanol as an esterifying agent. The fatty acid esters were isolated and analyzed on a gas chromatograph (Agilent, model 7890ª), coupled to a mass detector (Agilent 5975C), using a ZB-Wax Polyethylene Glycol column (30 m length × 0.25 mm inner diameter × 0.25 µm film thickness). Helium was used as a carrier gas, and the injection flow rate was 1 mL/min at a split ratio of 1:10. The initial column temperature was set at 50°C, maintained for 2 min, then raised to 220°C at a rate of 4°C/min, and maintained for 7 min. The injector temperature was 250°C. The identification of the fatty acids was performed by comparing the retention times of the methyl esters of the samples with those of authentic standards (Sigma).

2.6 Sensory evaluation of the protein bars

The sensory evaluation of the protein bars was performed after the microbiological characterization. The products were partitioned into 20 g per sample, individually packaged, and offered to 60 untrained assessors. A coded sample of each treatment was used, with three random identification digits. A glass of mineral water at room temperature and a form for the evaluation of the protein bars were also provided to the assessors. The attributes color, flavor, texture, aroma, and overall impression were evaluated on a hedonic scale of 9 points, with the scores ranging from 1 (disliked very much) to 9 (liked very much) (Dutcosky, 2011). The purchase intention test was also performed using a 5-point scale, in which 5 represented the maximum score "would certainly buy" and 1 represented the minimum score "would certainly not buy," as reported by Damásio and Silva (1996).

The acceptability index (AI) was calculated using Equation 1:

$$AI = A \times 100/B \quad (1)$$

Where:

A: average score for the product;

B: maximum score on the scale (Dutcosky, 2011; Teixeira et al., 1987).

2.7 Experimental design

A complete randomized design was performed with four treatments to evaluate the effect of the addition of FPC to protein bars with different flavors when compared with whey protein-based bars (New Millen®) with banana flavor. The results were analyzed by analysis of variance, and the means were compared by Tukey's test with a significance level of 5% using the software R Studio (version 1.2.1335).

The microbiological characterization and the fatty acid profile were determined only to characterize the product through descriptive analysis.

3 RESULTS AND DISCUSSION

3.1 Microbiological analysis of the fish protein concentrate and protein bars

The results of the microbiological analysis of the protein concentrates and protein bars, regardless of the treatment, were in accordance with the microbiological standards established by the Brazilian legislation (Brasil, 2001), RDC 12, from the National Health Surveillance Agency of the Ministry of Health, which are coliforms < 3 MPN/g, *Staphylococcus* counts < 1×10^2 CFU/g, and absence of *Salmonella*. The MPN of coliforms at 35 and 45°C, positive coagulase staphylococci counts in CFU/g, and absence of *Salmonella* spp. in 25 g of the sample showed that the products are fit for human consumption (Table 2).

Studies on the addition of fish waste flour and FPC to food formulations have shown suitable conditions for consumption, showing that the raw material was within the microbiological standards required by the current legislation (Brasil, 2019a, 2019b) for the production of a product for nutritional enrichment (Souza et al., 2017; Vitorino et al., 2020). Tilapia skin can also be used to produce flour or protein concentrate for inclusion in different types of foods, especially those with low protein or mineral levels, aimed at nutritional enrichment.

3.2 Characterization of the protein concentrate used in the protein bar formulations

The tilapia protein concentrate (FPC) presented 6.17% moisture, 75.16% crude protein, 17.27% lipids, 3.33% ash, and an energy value of 457.90 kcal/100 g. Rebouças et al. (2012) studied FPC from Nile tilapia filleting residues and reported 4.85, 85.00, 2.45, and 8.20% of moisture, protein, ash, and lipid contents, respectively. The differences when compared with this study are due to the methodology used, mainly for the lipid extraction. The authors performed delipidation and deodorization of the protein concentrate to reduce the fat content, and the lower moisture content led to an increase in crude protein.

The essential amino acids found in the FPC included lysine (8.05%), leucine (6.52%), isoleucine (3.97%), valine (3.76%), threonine (3.71%), and in smaller proportions phenylalanine (2.93%), methionine (2.24%), and tryptophan (0.46%). The non-essential amino acids found above 5% were glutamic acid (12.28%), aspartic acid (8.68%), and arginine (5.02%). The others were alanine (4.43%), serine (3.22%), glycine (3.02%), proline (2.61%), tyrosine (2.57%), histidine (1.64%), and cystine (0.83%). The FPC presented tryptophan as the limiting amino acid. In turn, the average amino acid composition of whey protein concentrate was 15.4 mg glutamic acid, 11.8 mg leucine, 10.7 mg aspartic acid, 9.5 mg lysine, 4.9 mg alanine, 4.7 mg isoleucine, 4.7 mg valine, 4.6 mg threonine, 4.2 mg proline, 3.9 mg serine, 3.8 mg asparagine, 3.4 mg glutamine, 3.4 mg tyrosine, 3.1 mg methionine, 3.0 mg phenylalanine, 2.4 mg arginine, 1.7 mg cysteine, 1.7 mg glycine, 1.7 mg histidine, and 1.3 mg tryptophan per gram of protein (Etzel, 2004). Therefore, the two types of protein products exhibited different amino acid profiles.

The tilapia protein concentrate (FPC) presented 27 fatty acids, and the oleic, palmitic, and linoleic fatty acids stood out in larger amounts, with values of 5.78, 4.52, and 2.20%, respectively.

Table 2. Microbiological analysis of fish protein concentrate (FPC), fish protein-enriched bars, and commercial whey protein-based bars with different flavors.

Microbiological characterization	Bars				FPC (Tilapia protein concentrate)
	Commercial protein complex – banana	Addition of FPC with flavor			
		Banana	Orange/apple	Peanut	
Coliforms (35°C) $MPNg^{-1}$	< 3	< 3	< 3	< 3	< 3
Coliforms (45°C) $MPNg^{-1}$	< 3	< 3	< 3	< 3	< 3
Positive coagulase staphylococci	< 10^2	< 10^2	< 10^2	< 10^2	< 10^2
Salmonella sp. 25 g	Absence	Absence	Absence	Absence	Absence

$MPNg^{-1}$: most probable number per gram; $CFUg^{-1}$: colony-forming units per gram.

The pH and Aw of the FPC were 6.08 and 0.13, respectively. This pH value is considered slightly acid, and the product has a very low Aw, contributing to preservation and preventing the development of microorganisms (Silva & Marsaioli Jr., 2003).

3.3 Water activity, pH, and instrumental color measurements of the protein bars

The water activity (Aw) and pH of foods are intrinsically related to preservation and are essential aspects for the evaluation of the shelf life of foods. The fish protein-enriched bars with banana and orange/apple flavors showed lower pH values, with an average value of 5.56 (Table 3). However, in general, all bars presented a slightly acid pH and were able to inhibit microbial growth, considering that the majority of microorganisms have their multiplication favored in pH around 6.5–7.0 (Jay, 2005).

Although Brazilian legislation establishes a maximum moisture of 15% for cereal products (Brasil, 2005), moisture is not a safe indicator to predict microbial activity and physicochemical reactions (Silva, 2008). In this sense, water activity is an indicator of water available for biochemical reactions and microbial growth (Silva, 2008). Also, Aw of a product is considered one of the most important parameters for the stability of food products. According to Silva and Marsaioli Jr. (2003), Aw values below 0.60 correspond to good stability against possible changes caused by microorganisms and other chemical reactions. Therefore, the bars of this study exhibited high Aw, which may favor the development of microorganisms during storage at room temperature (around 25°C). The peanut flavor bar with FPC inclusion presented a lower Aw (0.92) when compared with the other formulations, due to the lower moisture content of this ingredient. In turn, the FPC-enriched bars with banana and orange/apple flavors showed higher Aw due to the use of the fruits *in natura*. Therefore, there is a need for greater care during storage, which should be performed at lower temperatures, for greater protection and increased shelf life of the product regardless of the ingredient used as the flavoring agent.

Many food spoilage bacteria survive in temperatures below 10°C, with greater growth at temperatures between 15 and 20°C, while pathogenic bacteria that fall within the mesophilic bacteria have an optimum temperature between 30 and 37°C (Franco & Landgraf, 2008). Therefore, refrigeration or dehydration to reduce the moisture content and consequently the Aw is recommended for the bars of the present study.

The analysis of the instrumental color of protein bars is an important requirement for the consumer at the time of purchase. No significant difference ($p > 0.05$) (Table 3) was observed for the color parameters analyzed in this study, with L*, a*, and b* values of 35.66, 6.79, and 15.36, respectively, probably due to the chocolate topping used in all protein bars.

3.4 Proximate composition and energy value of the protein bars

Knowledge of the composition of foods is essential for the evaluation of nutrient bioavailability in the diet. In addition to the high water activity of the bars, the moisture content ranged from 15.76 to 16.59% (Table 4), which is above the recommended by legislation for cereal products. All FPC-enriched bars had significantly higher moisture when compared with the commercial whey-based bar. In addition, the ash content of the products increased with the addition of FPC when compared with the commercial bar. These findings corroborate the findings of Coradini et al. (2018) and Vitorino et al. (2020), who stated that FPCs increased the mineral contents of cereal bars.

Table 3. Aw, pH, and instrumental color of whey protein-based bars and FPC-enriched bars.

Parameters	Commercial protein complex Banana	Fish protein concentrate (FPC)			p-Value	CV (%)
		Banana	Orange/apple	Peanut		
Aw	0.92 ± 0.005[b]	0.94 ± 0.000[a]	0.94 ± 0.006[a]	0.92 ± 0.004[b]	0.000	0.59
pH	5.84 ± 0.13[a]	5.52 ± 0.02[c]	5.60 ± 0.03[bc]	5.97 ± 0.07[a]	0.001	1.59
Instrumental color						
L*	38.15 ± 2.45[a]	38.18 ± 4.50[a]	37.03 ± 3.59[a]	29.28 ± 2.28[a]	0.078	11.44
a*	6.58 ± 0.66[a]	6.62 ± 0.74[a]	6.93 ± 0.94[a]	7.03 ± 0.84[a]	0.921	14.45
b*	14.78 ± 0.47[a]	15.00 ± 0.65[a]	16.95 ± 0.84[a]	14.74 ± 1.32[a]	0.099	6.99

Values expressed as mean ± standard deviation followed by different letters in the same row indicate significant differences by Tukey's test ($p < 0.05$).

Table 4. Proximate composition of whey protein-based bars and FPC-enriched bars with different flavors.

Proximate composition (%)	Commercial protein complex	Fish protein concentrate (FPC)			p-Value	CV (%)
	Banana	Banana	Orange/apple	Peanut		
Moisture	13.59 ± 2.83[b]	16.25 ± 3.70[a]	16.59 ± 2.91[a]	15.76 ± 1.94[a]	0.626	19.21
Protein	19.62 ± 1.09	22.19 ± 1.42	21.94 ± 2.53	20.75 ± 1.16	0.258	9.04
Lipids	2.38 ± 0.18[b]	2.48 ± 0.25[b]	2.55 ± 0.09[b]	3.15 ± 0.45[a]	0.005	7.43
Ash	2.32 ± 2.05[b]	4.03 ± 1.70[a]	4.18 ± 1.66[a]	4.28 ± 1.35[a]	0.048	6.55
Carbohydrates	62.27 ± 1.31[a]	55.82 ± 0.66[b]	55.36 ± 0.74[b]	56.01 ± 1.80[b]	0.001	2.60
Energy value (kcal/100 g)	348.29 ± 0.90[a]	331.29 ± 0.90[c]	329.62 ± 0.43[c]	335.61 ± 0.10[b]	0.001	0.03

Values expressed as mean ± standard deviation followed by different letters in the same row indicate significant differences by Tukey's test ($p < 0.05$).

The addition of FPCs to the protein bar formulations led to an increase in protein availability. However, the protein contents in the bars varied greatly, with values of 9.76 and 10.27% for the formulations with the inclusion of 4.5 and 10% FPC, respectively (Souza et al., 2017; Vitorino et al., 2020).

Although no significant differences were observed in the protein levels between the bars, the average protein content was high (21.12%) when compared with the protein contents of the commercial bar (4.4%) (Srebernich et al., 2016). However, protein bars should meet the standards established by the RDC 18, April 2010 of the National Health Surveillance Agency (ANVISA, 2010), which states that to be called a protein bar it must contain at least 10 g of protein per serving, which is much lower than the results of this experiment (Table 4), with 21.12% of crude protein. This variation in protein content is due to several factors, including the type of raw material, variety, and quantities of ingredients used in the protein bar formulations.

The carbohydrate contents and the energy value were reduced significantly with the inclusion of FPC. The carbohydrate levels of the whey protein-based bars and FPC-enriched bars were 62.27 and 55.36%, respectively, with a reduction of 11.09% in the carbohydrate content of the FPC-enriched product.

Regarding the energy value, a significant reduction was observed for the FPC-enriched bars, and the banana- and orange/apple-flavored bars showed significantly lower energy value, with a reduction of 5.37 kcal/100 g. Vitorino et al. (2020) studied cereal bars made with tilapia protein concentrate and reported 55.08% and 372.18 kcal/100 g of carbohydrates and energy value, respectively. On the contrary, Souza et al. (2017) observed no reduction in carbohydrate contents and energy value of bars made with 4.5% protein concentrate. This result is due to the inclusion level of FPC, as Vitorino et al. (2020) used 10% and Souza et al. (2017) used 4.5% of FPC.

The lipid content of the protein bars of this experiment was significantly higher (3.15%) for the peanut-flavored bars, probably due to the amount of this ingredient that contains high fat levels. Freitas and Moretti (2006) reported that conventional cereal bars usually have fat levels between 4 and 12%. In this study, the protein bars showed fat levels around 2.48–3.15%, regardless of the treatment with the inclusion of FPC, probably due to the types of ingredients used in the formulations.

When fish is used in a food formulation, besides the lipid content, the lipid profile should also be considered because fish has essential fatty acids that are important for human nutrition, such as polyunsaturated fatty acids and low LDL cholesterol, which act in the prevention of cardiovascular diseases (Vila Nova et al., 2005). LDL is a low-density lipoprotein and carries cholesterol from the liver and intestine to the tissue cells. It is considered bad cholesterol because high LDL levels accumulate in the inner walls of the arteries (Drewnowski & Specter, 2004; Goldstone et al., 2001; Nicklas et al., 2004).

3.5 Fatty acids profile of the protein bars

A total of 21 fatty acids (Table 5) were detected in the protein bars of this study, including palmitic acid, palmitoleic

Table 5. Fatty acid profile of whey protein-based bars and FPC-enriched bars with different flavors.

Fatty acids (%)	**Commercial protein complex**	**Fish protein concentrate (FPC)**		
	Banana	**Banana**	**Orange/apple**	**Peanut**
Caprylic acid	2.3 ± 0.003	1.82 ± 0.017	1.98 ± 0.011	0.55 ± 0.022
Lauric acid	5.34 ± 0.007	2.11 ± 0.011	4.32 ± 0.008	1.92 ± 0.015
Myristic acid	3.88 ± 0.001	1.56 ± 0.026	0.98 ± 0.006	2.38 ± 0.012
Myristoleic acid	0.12 ± 0.021	0.08 ± 0.017	0.10 ± 0.013	0.14 ± 0.005
Penta-decanoic acid	0.23 ± 0,036	0.33 ± 0.007	0.13 ± 0.080	0.42 ± 0.010
Palmitic acid	22.11 ± 0.018	28.44 ± 0.037	30.84 ± 0.016	27.81 ± 0.003
Palmitoleic acid	0.21 ± 0.003	0.82 ± 0.010	1.43 ± 0.001	0.87 ± 0.037
Margaryc acid	0.33 ± 0.012	0.43 ± 0.011	0.27 ± 0.002	0.21 ± 0.018
Cis-10-	0.76 ± 0.013	1.36 ± 0.002	1.35 ± 0.004	2.54 ± 0.009
Heptadecenoic acid	4.55 ± 0.019	7.05 ± 0.015	5.93 ± 0.011	3.25 ± 0.005
Stearic acid	31.81 ± 0.005	32.44 ± 0.032	30.29 ± 0.013	40.93 ± 0.021
Oleic acid	1.29 ± 0.036	0.12 ± 0.006	1.32 ± 0.032	1.34 ± 0.011
Vaccinic acid	7.43 ± 0.025	9.19 ± 0.005	9.55 ± 0.003	11.68 ± 0.023
Linoleic acid	3.97 ± 0.017	3.77 ± 0.011	3.56 ± 0.013	8.43 ± 0.008
Linolenic acid	1.20 ± 0.028	0.27 ± 0.013	0.31 ± 0.026	0.99 ± 0.026
CLA (conjugated linoleic acid)	0.44 ± 0.011	0.66 ± 0.018	0.16 ± 0.011	0.21 ± 0.012
Eicosanoic acid	0.81 ± 0.012	0.33 ± 0.021	0.23 ± 0.017	0.30 ± 0.010
Gondoic acid	0.19 ± 0.017	0.76 ± 0.031	0.07 ± 0.021	0.09 ± 0.026
Eicosadienoic acid	0.22 ± 0.026	0.13 ± 0.017	0.12 ± 0.002	0.23 ± 0.012
Eicosatrienoic acid	0.50 ± 0.034	0.33 ± 0.010	0.32 ± 0.003	0.41 ± 0.019
Arachidonic acid	0.24 ± 0.011	0.89 ± 0.011	0.44 ± 0.011	0.31 ± 0.014

Values are expressed as mean ± standard deviation. Analysis performed in triplicate.

acid, cis-10-heptadecenoic acid, acid behenic, and linoleic acid, which is an essential fatty acid and polyunsaturated biocursors of the omega 3 and 6 family (Martin et al., 2006). In addition, the peanut-flavored protein bars stood out for the fatty acids linoleic (11.68%), linolenic (8.43%), and especially oleic (40.93%), which was higher than all other fatty acids. Peanut is known as an excellent plant source of mono- and polyunsaturated acids, a characteristic that influences its nutritional quality (Win et al., 2011).

Fatty acids considered atherogenic (lauric and myristic) were found in larger amounts in the commercial whey protein-based bars. In excess, they may increase the risk of cardiovascular disease with effects similar to trans fats (Lottenberg, 2009). Although some fatty acids were observed in higher abundance for the bars with different flavors, characteristic of each ingredient used, the quality of the fatty acid profile of the FPC-enriched bars is remarkable, thus proving to be a potential product as a source of different fatty acids.

3.6 Sensory evaluation of the protein bars

The inclusion of FPC did not interfere with the sensory aspects of the protein bars (Table 6), with mean scores of 7.51, 7.59, 6.63, 7.17, and 7.3 for the attributes color, aroma, texture, flavor, and overall impression, respectively. Protein concentrates from different fish species have been studied and presented satisfactory results for the supplementation of cereal bars (Souza et al., 2017, Vitorino et al., 2020) and other products such as breakfast cereal (Souza et al., 2021), cookies (Souza et al., 2022), alfajor (Kimura et al., 2017), and cakes (Costa et al., 2022; Goes et al., 2016; Lazzari et al., 2021) with the purpose of nutritional enrichment.

FPC presents typical organoleptic characteristics; thus, the acceptance of products supplemented with FPC can vary according to the formulation and ingredients that can mask the fish flavor. In this study, the dark chocolate coating may have affected the sensory aspect of the product because fish flavor or aroma was not reported by consumers.

This study evaluated the total replacement of a protein source by FPC. The results were satisfactory, considering that the inclusion of FPC was 5.30% of the total formulation, with no significant difference between the sensory attributes, as well as the purchase intention of the product (Table 6). The banana-flavored bars exhibited high texture and aroma scores, ranging from 6.39 to 8.03, respectively.

Souza et al. (2017) studied protein bars with the inclusion of 4.5% of tilapia protein concentrate and reported lower scores when compared with this study, with maximum scores of 6.80 for the attribute color. However, for purchase intention, the bar made with tilapia protein concentrate showed a score of 4.02, which is similar to the average score obtained in this experiment for this parameter (Table 6). Vitorino et al. (2020) analyzed the sensory attributes of bars with the inclusion of 10% protein concentrate from different fish species and reported scores ranging from 5.70 to 6.68, with the highest scores observed for the attribute color, although those bars did not receive chocolate topping. The chocolate topping provides an increase in sensory scores, increasing the product's AI.

No significant differences ($p > 0.05$) in color were observed for the products in the sensory study and the instrumental color analysis of the parameters L*, a*, and b*, demonstrating similar perceptions for the analysis with humans as the instrumental analysis (Tables 3 and 6). The products exhibited high sensory scores, ranging from 7.32 to 7.61, which correspond to liked moderately (7) to liked very much (8) (Dutcosky, 2011).

The coproducts from Nile tilapia, especially flour and protein concentrates, have shown promising results, especially in sweets, such as cakes, alfajor, cookies, crackers, and cereal bars (Franco et al., 2013; Fuzinatto et al., 2015; Goes et al., 2016; Kimura et al., 2017; Souza et al., 2017), provided that their inclusion does not exceed 15% in the formulation, once larger inclusions may interfere with the acceptance of the products.

A fundamental aspect of the evaluation of a product aimed at the consumer market is the determination of the AI, and values ≥ 70% are recommended for a product to be considered accepted (Dutcosky, 2011; Moscatto et al., 2004; Teixeira et al, 1987). As shown in Table 7, all products had AI values of > 70% for all attributes evaluated, making them suitable for marketing. Only the attribute texture of the banana-flavored bar had a lower AI but was still within the recommended acceptable value. When analyzing the AI of the protein bars for all attributes, the FPC-enriched bars with orange/apple flavor had the highest AI (83.45%), while the others were also well accepted by consumers because they showed AI > 70%.

However, further studies are required to evaluate a higher inclusion level of FPC in protein bars of different flavors (banana, orange/apple, and peanut), once no significant difference was observed in this study for some parameters analyzed, except

Table 6. Sensory evaluation and purchase intention of whey protein-based bars and FPC-enriched bars with different flavors.

Attributes	Commercial protein complex	Fish protein concentrate			p-Value	CV (%)
	Banana	Banana	Orange/apple	Peanut		
Color	7.61 ± 1.38	7.52 ± 1.66	7.58 ± 1.50	7.32 ± 1.69	0.891	21.18
Aroma	7.39 ± 1.16	8.03 ± 1.03	7.77 ± 1.28	7.16 ± 1.65	0.051	17.43
Texture	6.52 ± 1.72	6.39 ± 1.76	7.00 ± 1.74	6.61 ± 1.90	0.575	27.21
Flavor	6.90 ± 1.89	7.51 ± 1.50	7.52 ± 1.66	6.77 ± 2.03	0.225	25.23
Overall impression	7.13 ± 1.98	7.58 ± 1.29	7.68 ± 1.20	6.81 ± 1.84	0.133	22.47
Purchase intention	3.90 ± 1.12	3.93 ± 1.04	4.16 ± 0.95	3.68 ± 1.40	0.442	29.6

Values are expressed as mean ± standard deviation.

Table 7. Acceptability index (AI %) of whey protein-based bars and FPC-enriched bars with different flavors.

Attributes	Commercial protein complex	Fish protein concentrate bar		
	Banana	Banana	Orange/apple	Peanut
Color	84.58	83.51	84.29	81.36
Aroma	82.08	89.25	86.38	79.57
Texture	72.40	70.97	77.78	73.48
Flavor	76.71	83.51	83.51	75.27
Overall impression	79.21	84.23	85.30	77.06
AI (%)	78.99	82.29	83.45	77.35

for the peanut flavored bar, which showed a higher fat content. This strategy of higher addition of concentrate can lead to higher nutritional efficiency, once it can increase the protein content of the bars.

The FPC-enriched bars of this study, regardless of the flavor, showed promising results, both concerning consumer acceptance and the proximate composition, with high protein, ash contents, quality fatty acids, lower carbohydrate content, and lower energy value. In turn, the moisture and consequently the Aw of the formulations should be reduced.

4 CONCLUSIONS

The FPC-enriched bars with different flavors showed promising results regarding the nutritional parameters, fatty acids profile, and sensory properties, allowing the total replacement of commercial protein complexes by this fish coproduct.

The use of peanuts in the manufacture of protein bars led to an improvement in the fatty acid profile, thus proving to be a potential ingredient.

The bars presented high water activity values, thus requiring the use of preservatives for large-scale production and allowing better conservation and a longer shelf life of the product. Dehydration of the protein bars before the chocolate coating is required to reduce the moisture contents and Aw of the product.

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