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DEVELOPMENT AND CHARACTERIZATION OF WHOLE WHEAT BREAD ENRICHED WITH FISH PROTEIN CONCENTRATE AND NATURAL PRESERVATIVES

DESENVOLVIMENTO E CARACTERIZAÇÃO DE PÃO INTEGRAL ENRIQUECIDO COM CONCENTRADO PROTEICO DE PEIXE E CONSERVANTES NATURAIS

DESARROLLO Y CARACTERIZACIÓN DE PAN INTEGRAL ENRIQUECIDO CON CONCENTRADO PROTEICO DE PESCADO Y CONSERVANTES NATURALES

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Abstract

The use of by-products from the fishing industry in human food constitutes a strategy aligned with the principles of the circular economy and food security. This study aimed to develop and characterize a whole wheat bread enriched with fish protein concentrate (FPC), using propolis and chitosan as natural preservatives to replace calcium propionate. Three formulations were prepared: a control (without FPC) and two formulations with partial replacement of whole wheat flour by 1.5% (T15) and 3% (T30) of FPC. Initially, all formulations were subjected to microbiological analyses and sensory evaluation. Based on the sensory results, the formulation with the highest acceptance, along with the control formulation, was selected for analyses of proximate composition, yield, water activity, colorimetric parameters, and lipid peroxidation (TBARS). The microbiological results indicated compliance with current legislation, demonstrating the product's safety. In the sensory analysis, no statistically significant differences were observed among the formulations for the evaluated attributes. However, formulation T30 showed an acceptability index (AI) ≥ 70%, the highest among the enriched formulations. This formulation showed approximately 25% increase in protein content compared to the control, without significant differences in moisture, lipid, and carbohydrate contents, or in technological parameters, water activity, instrumental color, and oxidative stability. It is concluded that whole wheat bread enriched with 3% FPC and preserved with propolis and chitosan is technologically viable, microbiologically safe, and sensorially acceptable, configuring itself as a nutritious and sustainable alternative for the valorization of by-products from the fishing industry.

Keywords: Fish protein concentrate; whole wheat bread; natural preservatives; propolis; chitosan.

Resumo

O aproveitamento de subprodutos da indústria pesqueira na alimentação humana constitui uma estratégia alinhada aos princípios da economia circular e da segurança alimentar. Este estudo teve como objetivo desenvolver e caracterizar um pão integral enriquecido com concentrado proteico de peixe (CPP), utilizando própolis e quitosana como conservantes naturais em substituição ao propionato de cálcio. Foram elaboradas três formulações: controle (sem CPP) e duas formulações com substituição parcial da farinha de trigo integral por 1,5% (T15) e 3% (T30) de CPP. Inicialmente, todas as formulações foram submetidas a análises microbiológicas e à avaliação sensorial. Com base nos resultados sensoriais, a formulação com maior aceitação, juntamente com a formulação controle, foi selecionada para análises de composição proximal, rendimento, atividade de água, parâmetros colorimétricos e peroxidação lipídica (TBARS). Os resultados microbiológicos indicaram conformidade com a legislação vigente, evidenciando a segurança do produto. Na análise sensorial, não foram observadas diferenças estatisticamente significativas entre as formulações nos atributos avaliados. Entretanto, a formulação T30 apresentou índice de aceitabilidade (IA) ≥ 70%, sendo a mais bem avaliada entre as formulações enriquecidas. Essa formulação apresentou um incremento de aproximadamente 25% no teor proteico em relação ao controle, sem diferenças significativas nos teores de umidade, lipídios e carboidratos, bem como nos parâmetros tecnológicos, atividade de água, cor instrumental e estabilidade oxidativa. Conclui-se que o pão integral enriquecido com 3% de CPP e conservado com própolis e quitosana é tecnologicamente viável, microbiologicamente seguro e sensorialmente aceito, configurando-se como uma alternativa nutritiva e sustentável para a valorização de subprodutos da indústria pesqueira.

Palavras-chave: Concentrado proteico de peixe; pão integral; conservantes naturais; própolis; quitosana.

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Resumen

El uso de subproductos de la industria pesquera en la alimentación humana constituye una estrategia alineada con los principios de la economía circular y de la seguridad alimentaria. Este estudio tuvo como objetivo desarrollar y caracterizar un pan integral enriquecido con concentrado de proteína de pescado (CPP), utilizando propóleo y quitosano como conservantes naturales para reemplazar el propionato de calcio. Se prepararon tres formulaciones: un control (sin CPP) y dos formulaciones con reemplazo parcial de harina integral por 1,5% (T15) y 3% (T30) de CPP. Inicialmente, todas las formulaciones fueron sometidas a análisis microbiológicos y evaluación sensorial. Con base en los resultados sensoriales, la formulación con mayor aceptación, junto con la formulación control, se seleccionó para los análisis de composición proximal, rendimiento, actividad de agua, parámetros colorimétricos y peroxidación lipídica (TBARS). Los resultados microbiológicos indicaron el cumplimiento de la legislación vigente, lo que demuestra la seguridad del producto. En el análisis sensorial no se observaron diferencias estadísticamente significativas entre las formulaciones en los atributos evaluados. Sin embargo, la formulación T30 mostró un índice de aceptabilidad (IA) ≥ 70%, lo que la convirtió en la mejor evaluada entre las formulaciones enriquecidas. Esta formulación mostró un aumento de aproximadamente el 25% en el contenido proteico en comparación con el control, sin diferencias significativas en el contenido de humedad, lípidos y carbohidratos, así como en los parámetros tecnológicos, la actividad del agua, el color instrumental y la estabilidad oxidativa. Se concluye que el pan integral enriquecido con 3% de CPP y conservado con propóleo y quitosano es tecnológicamente viable, microbiológicamente seguro y sensorialmente aceptable, lo que lo convierte en una alternativa nutritiva y sostenible para la valorización de subproductos de la industria pesquera.

Palabras clave: Concentrado proteico de pescado; pan integral; conservantes naturales; propóleo; quitosano.

1. Introduction

The growing demand for foods that combine high nutritional value, health benefits, and sustainable production systems has driven the development of innovative food products in the last decade (Bigliardi; Galati, 2013). In this context, bakery products, especially whole-wheat breads, stand out as strategic technological matrices for the incorporation of functional ingredients, given their widespread consumption, sensory acceptability, and formulation versatility (Melini et al., 2020). Simultaneously, the adoption of circular economy principles has encouraged the valorization of agro-industrial by-products, transforming waste into high-value-added ingredients for human food (Ligarda-Samanez *et al*., 2025).

The fishing industry generates large volumes of solid waste, such as heads, bones, and trimmings, which, when improperly disposed of, pose a significant environmental and economic liability (Coppola *et al*., 2021). However, these by-products have high nutritional potential, being rich in high-biological-value proteins, minerals, and omega-3 polyunsaturated fatty acids (Silva *et al.*, 2014). In this

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scenario, processing these wastes in the form of fish protein concentrate (FPC) emerges as a sustainable and technically viable alternative, allowing for the reduction of environmental impacts and the obtaining of functional ingredients applicable to the development of fortified foods (Coppola *et al*., 2021; Rana *et al*., 2023).

Despite the nutritional benefits, incorporating fish-derived ingredients into baked goods presents significant technological and sensory challenges. Alterations in texture, color, and sensory profile, especially those related to the characteristic odors and flavors of fish, can compromise consumer acceptance (Abraha *et al*., 2018). Furthermore, the higher unsaturated lipid content in these ingredients can promote lipid oxidation, reducing the stability and shelf life of the final product (Dragoev, 2024). Therefore, technological strategies that minimize these effects are fundamental to the viability of baked goods enriched with fish protein.

Concomitantly, there is a growing consumer demand for foods formulated with less synthetic additives, driving the search for preservatives of natural origin that meet clean label trends (Munekata *et al*., 2020). In this context, propolis and chitosan stand out as promising natural compounds. Propolis, a resinous substance produced by bees, has recognized antimicrobial and antioxidant activity, mainly attributed to the presence of phenolic compounds and flavonoids (Pobiega; Kraśniewska; Gniewosz, 2019). Chitosan, in turn, is a biopolymer obtained from chitin, widely studied for its antimicrobial and antioxidant properties and its ability to form edible films that act as a barrier to food spoilage (Mesgari; Aalami; Sahebkar, 2021).

Although several studies report the isolated use of fish proteins or natural preservatives in bakery products, research evaluating, in an integrated way, the application of fish protein concentrate associated with the replacement of synthetic preservatives with natural compounds, simultaneously considering the microbiological, sensory, nutritional, and technological aspects of the final product, is still limited. This scientific gap underscores the need for studies exploring innovative and sustainable solutions for the baking industry.

Given the above, the present study aimed to develop and characterize whole wheat bread enriched with fish protein concentrate, using propolis and chitosan as

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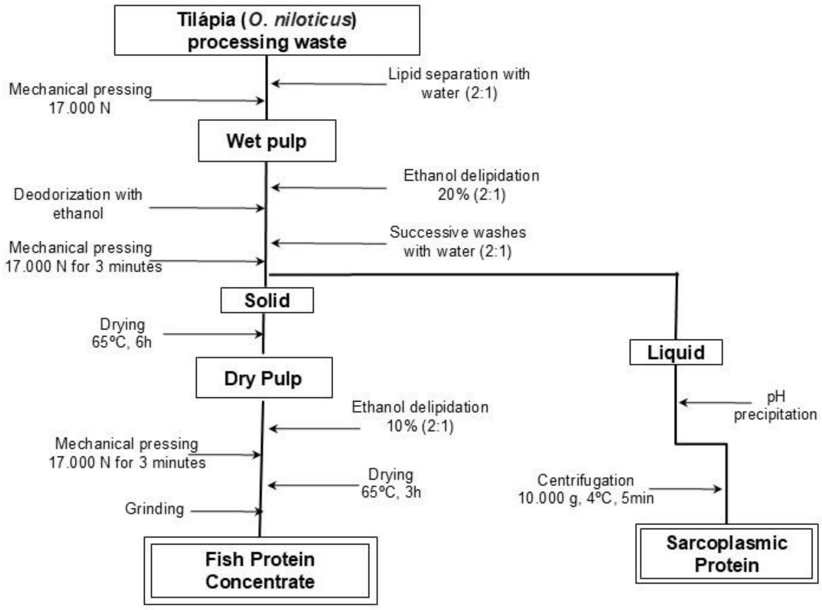
natural preservatives to replace calcium propionate, and to evaluate its microbiological quality, sensory acceptance, proximate composition, and technological properties.

2. Methodology

2.1. Obtaining the raw material

Fish protein concentrate (FPC) was obtained from tilapia (*Oreochromis niloticus*) trimmings, following the methodology described by Pereira *et al.* (2022), as shown in the flowchart in Figure 1. The trimmings were subjected to successive washes with water and ethanol, aiming to remove lipids and reduce the characteristic odor and taste of the fish, thereby concentrating the protein fraction.

Figure 1. Flowchart for obtaining fish protein concentrate.



Source: Elaborated by the authors (2024).

The proximate composition of FPC (moisture, crude protein, lipids, and ash) was determined according to the official methods of the Association of Official Analytical Chemists (AOAC, 1990), with the results presented in Table 1.

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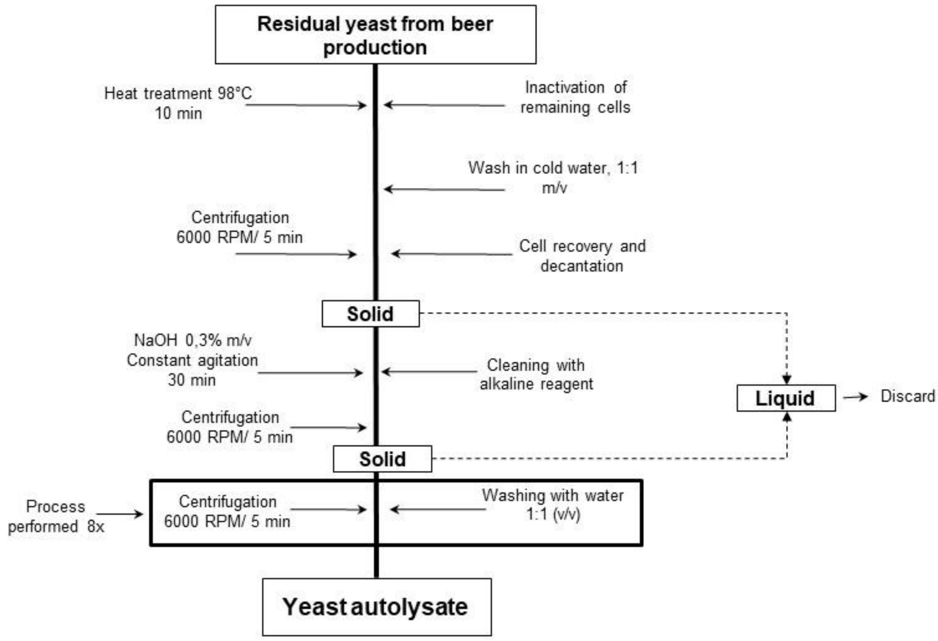
Table 1. Proximate composition of the fish protein concentrate (FPC) used in the production of the breads.

Parameters	Percentage (%)
Moisture	4.02 ± 0.01
Crude protein	90.01 ± 0.01
Lipids	6.47 ± 0.17
Ash	0.88 ± 0.01
Total caloric value	418.27 ± 0.90

Source: Elaborated by the authors (2025).

The residual yeast samples were provided by a craft brewery, and the yeast autolysate was obtained according to the procedure described by Pinto (2011), as illustrated in Figure 2. Chitosan, propolis extract, fructooligosaccharide (FOS), calcium propionate, and the other ingredients used in the formulations were commercially sourced and used according to the manufacturers′ specifications, without prior processing.

Figure 2. Flowchart of yeast autolysate preparation.



Source: Elaborated by the authors (2025).

2.2. Formulation and preparation of breads

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Three whole wheat bread formulations were developed, as described in Table 2: a control formulation, without the addition of fish protein concentrate (FPC), and two experimental formulations, named T15 and T30, in which whole wheat flour was partially replaced by FPC in proportions of 15 g kg^{-1} (1,5%) and 30 g kg^{-1} (3%), respectively.

In the enriched formulations (T15 and T30), the salt and sugar content was reduced by 50%, being partially replaced by yeast autolysate (1.5 g kg^{-1}) and fructooligosaccharides (15 g kg^{-1}), respectively. Furthermore, calcium propionate was replaced with propolis (2 g kg^{-1}) and chitosan (2 g kg^{-1}), while the other ingredients and the total formulations weight remained constant.

The bread was prepared by individually weighing the ingredients on an analytical balance. The water was preheated to 35 °C, then added to the dry yeast and sugar, and left to rest for 15 minutes to activate the yeast. Then, the eggs, olive oil, red propolis extract, chitosan, yeast autolysate, FPC, and whole wheat flour were added. The mixture was homogenized by kneading manually for approximately 5 minutes and left to rest for 40 minutes for fermentation. Subsequently, the bread was baked in a preheated oven at 180 °C for 40 minutes.

Table 2. Ingredients used in the formulations of whole-wheat breads enriched with fish protein concentrate.

Ingredients (kg^{-1})	Control	T15	T30
Whole wheat flour	500	485	470
FPC	-	15	30
Yeast autolysate	-	1.5	1.5
White sesame seeds	15	15	15
NaCl	10	5	5
Dry yeast	6	6	6
Olive oil	50	50	50
Eggs	90	90	90
FOS	-	15	15
Sugar	30	15	15
Water (35 ºC)	300	300	300
Chitosan	-	2	2

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Propolis	-	2	2
Calcium propionate	0.5	-	-
Total	1001.5	1001.5	1001.5

FPC: fish protein concentrate; NaCl: sodium chloride; FOS: fructooligosaccharides.
Source: Elaborated by the authors (2025).

2.3. Microbiological characterization

Microbiological analyses were performed to determine coliforms at 45 °C, coagulase-positive staphylococci, *Salmonella* spp., and molds and yeasts, according to the methodologies described in the American Public Health Association (APHA, 2015). Analyses were performed on all formulations produced.

2.4. Sensory analysis

The sensory evaluation was conducted in a fitness center, with 30 randomly selected, untrained tasters aged 18 or older who signed the Informed Consent Form. The study was approved by the Research Ethics Committee of the Federal University of Recôncavo da Bahia, under opinion number 3.362.931. Before the evaluation, participants received instructions on the test procedure. To cleanse the palate, sparkling mineral water and water crackers were provided between samples. The samples were served on disposable plates, each coded with a random number.

The attributes of flavor, aroma, color, texture, mouthfeel, and global acceptance were evaluated using a nine-point hedonic scale, ranging from 1 (extremely disliked) to 9 (extremely liked), as described by Stevanato *et al.* (2007). The consumption attitude and purchase intention were evaluated using a seven-point scale ranging from 1 (lowest attitude/intention) to 7 (highest attitude/intention).

The acceptability index (AI) was calculated using the equation: AI (%) = (X/Y) x 100, where X is the average of the scores assigned to the product and Y is the maximum score on the scale. AI values ≥ 70% were considered indicative of good sensory acceptance (Dutcosky, 1996).

Following sensory analysis, the formulation with the highest sensory acceptance, along with the control sample, was selected for proximate composition analysis and technological characterization.

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2.5. Proximate composition of breads

The proximate composition of the control formulation and the formulation selected in the sensory analysis was determined by analyzing moisture, crude protein, lipids, ash, and carbohydrates using the official AOAC methods (AOAC, 1990).

2.6. Technological analysis of breads

2.6.1. Yield

The yield of the bread (%) was determined by the ratio between the weight of the dough before baking and the weight of the product after baking.

2.6.2. Water activity

Water activity (Aw) was measured using a Nov-Labstart model water activity meter (Tecnal®, São Paulo, Brazil).

2.6.3. Colorimetric analysis

Instrumental color was determined using a CR-400 colorimeter (Konica Minolta®, Tokyo, Japan), with readings of the parameters L* (luminosity), a* (variation from green to red) and b* (variation from blue to yellow), according to the CIE (Commission Internationale de l'Éclairage) system.

2.6.4. Analysis of thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was evaluated by quantifying thiobarbituric acid-reactive substances (TBARS) according to the method of Buege and Aust (1978). The results were expressed as µmol malondialdehyde equivalents (MDA) kg^{-1} of dry sample.

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2.7. Statistical analysis

The data were subjected to normality (Shapiro-Wilk) and homogeneity of variances (Levene) tests. Once the assumptions were met, the results were expressed as mean ± standard deviation and subjected to analysis of variance (ANOVA). When significant differences were observed, the means were compared using Tukey's test, at the 5% significance level ($p < 0.05$). Statistical analyses were performed using R software, in an RStudio environment (Version 2025.09.2).

3. RESULTS

3.1. Microbiological characterization

The results of the microbiological analyses of the whole wheat bread formulations are presented in Table 3. All samples evaluated (control, T15, and T30) met the criteria established by Normative Instruction No. 60, of December 23, 2019 (Brazil, 2019).

Table 3. Microbiological quality of whole wheat breads enriched with fish protein concentrate.

Microorganisms	Control	T15	T30	Legislation
Coliforms at 45 ºC	< 3.0	<3.0	<3.0	10^2 g^{-1} MPN g^{-1}
Salmonella spp.	Absence	Absence	Absence	Absence
Coag. positive *Staphylococcus*	< 10	< 10	< 10	≤ 10^3 CFU g^{-1}
Molds and yeasts	< 10	< 10	< 10	≤ 10^4 CFU g^{-1}

T15 = 1,5% FPC. T30 = 3% FPC. CFU = colony-forming unit. MPN = Most Probable Number.
Source: Elaborated by the authors (2025).

The formulations showed coliform at 45 ºC counts below 3.0 MPN g^{-1}, as well as the absence of *Salmonella* spp. in 25 g of product. No coagulase-positive *Staphylococcus*, molds, or yeasts were detected in the analyzed samples, demonstrating adequate hygiene conditions during processing and the production of microbiologically safe products.

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3.2. Sensory analysis

The results of the sensory analysis of the breads enriched with fish protein concentrate (FPC) are presented in Table 4. No statistically significant differences were observed between the formulations for the individual sensory attributes evaluated, including flavor, aroma, color, texture, mouthfeel, and global acceptance (p > 0.05), indicating that the addition of FPC, at the levels studied, did not promote perceptible changes capable of sensorially differentiating the products.

Despite no statistical differences, the descriptive analysis of medians showed high sensory acceptance of the T30 formulation, with scores predominantly in the "8 - I liked it very much" category for flavor, aroma, texture, and overall acceptance. For the acceptance and purchase intention tests, the T30 formulation presented medians of 6 ("I would eat it whenever I had the opportunity") and 5 ("I would buy it frequently"), respectively.

Table 4. Means and standard deviations of sensory attributes of whole wheat breads enriched with fish protein concentrate.

Attributes	Control		T15		T30		*p*-valor
	Mean	Med.	Mean	Med.	Mean	Med.	
Flavor	7.0 ± 1.5	7	6.3 ± 1.8	7	7.2 ± 1.9	8	0.107
Aroma	6.9 ± 1.6	7.5	6.4 ± 1.8	6.5	6.9 ± 1.9	8	0.492
Color	7.1 ± 1.6	7	7.1 ± 1.5	7	7.2 ± 1.3	7	0.896
Texture	7.0 ± 1.8	8	6.6 ± 1.7	7	7.3 ± 1.5	8	0.319
Mouthfeel	6.9 ± 1.7	7	6.0 ± 2.1	6	6.9 ± 1.9	7.5	0.156
Global acceptance	6.9 ± 1.5	7	6.4 ± 1.9	6	7.3 ± 1.7	8	0.126
Attitude test	4.1 ± 1.7	5	4.1 ± 1.6	4.5	4.6 ± 2.0	6	0.479
Purchase intention	4.7 ± 1.8	4	4.3 ± 1.9	4	5.1 ± 2.0	5	0.283
Acceptance index (%)	82.2 ± 5.0^{a}		69.4 ± 9.5^{b}		79.4 ± 7.7^{ab}		0.030

Med. = median. T15 = 1.5% FPC. T30 = 3% FPC. Means followed by different letters differ significantly ($p < 0.05$).

Source: Elaborated by the authors (2025).

When observing overall acceptance individually (Figure 3), a percentage greater than 76% was found, indicating that the addition of FPC to whole-grain

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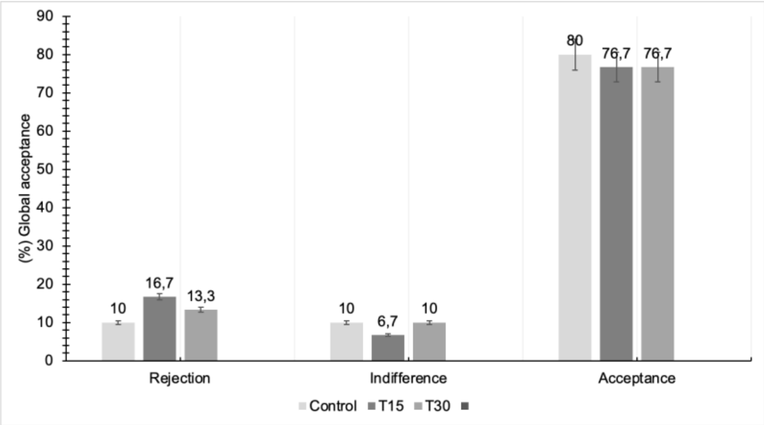
products resulted in high levels of acceptance, considering the scores assigned by the evaluators above the central value of the hedonic scale ("5 - indifferent").

Figure 3. Percentages of approval, indifference, and rejection of whole-wheat breads enriched with fish protein concentrate.



Source: Elaborated by the authors (2024).

The acceptability index showed a significant difference between the formulations ($p < 0.05$) (Table 4), with formulation T30 statistically positioned intermediately between the control and T15. Based on the sensory analysis results, formulation T30, along with the control, was selected for the proximate composition and technological characterization analyses.

3.3. Proximal composition

The proximate composition of the control formulation and the T30 formulation is presented in Table 5. A significant increase ($p < 0.05$) was observed in the crude protein content of the enriched bread (T30) compared to the control.

The ash content was significantly lower in the T30 formulation compared to the control ($p < 0.001$). For moisture, lipid, carbohydrate, and total caloric content, no statistically significant differences were observed between the evaluated formulations ($p > 0.05$).

Table 5. Proximate composition of control whole wheat bread and whole wheat bread enriched with fish protein concentrate (T30).

Parameters (%)	Control	T30	p-valor

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Moisture	6.46 ± 0.18	6.58 ± 0.03	> 0.05
Crude protein	14.99 ± 0.08	18.74 ± 1.14*	< 0.05
Lipids	15.13 ± 0.81	15.39 ± 0.92	> 0.05
Carbohydrates	60.76 ± 0.99	57.21 ± 2.14	> 0.05
Ash	2.67 ± 0.03*	2.24 ± 0.09	< 0.001
Total caloric value	439.15 ± 3.23	441.67 ± 4.12	> 0.05

T30 = 3% FPC. The presence of an asterisk in the lines indicates a significant difference according to the t-test ($p < 0.05$).

Source: Elaborated by the authors (2025).

3.4. Technological analysis of breads

The results regarding the technological parameters of the control bread and the T30 formulation are presented in Table 6. No significant differences ($p > 0.05$) were observed between the formulations regarding yield, water activity, and lipid peroxidation levels, expressed as malondialdehyde (MDA).

Table 6. Technological and instrumental color parameters of the control wholemeal bread and the formulation enriched with fish protein concentrate (T30).

Parameters	**Control**	**T30**	***p*-valor**
Yield (%)	93.75 ± 2.72	93.32 ± 4.31	> 0.05
Water activity	0.90 ± 0.05	0.91 ± 0.01	> 0.05
MDA (µmol mg^{-1})	4.54 ± 0.01	4.57 ± 0.01	> 0.05
Instrumental color	**Control**	**T30**	***p*-valor**
L *	53.75 ± 0.54	51.02 ± 0.35	> 0.05
a *	7.53 ± 0.78	7.82 ± 0.18	> 0.05
b *	21.76 ± 0.16	22.15 ± 3.67	> 0.05

T30 = 3% FPC. The absence of an asterisk in the rows indicates that there is no significant difference according to the t-test ($p < 0.05$). Results are expressed as mean ± standard deviation.

Source: Elaborated by the authors (2025).

Similarly, the instrumental color parameters (L*, a*, and b*) did not differ significantly between the control and T30 samples ($p > 0.05$), indicating similar chromatic behavior between the formulations.

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4. DISCUSSION

The microbiological conformity observed in all evaluated formulations demonstrates the adequacy of hygienic and sanitary conditions during processing and the effectiveness of the heat treatment applied during baking. The absence of *Salmonella* spp., coagulase-positive staphylococci, molds and yeasts, as well as the low counts of thermotolerant coliforms, indicates that the breads fully met the criteria established by current Brazilian legislation for bakery products (Brazil, 2019). These results corroborate the importance of adopting good manufacturing practices, including appropriate thermal processes, ensure the safety of food for consumption (Seixas; Muttoni, 2020).

Furthermore, maintaining microbiological quality even after replacing calcium propionate with propolis and chitosan demonstrates the potential of these compounds as natural preservatives. Propolis exhibits broad-spectrum antimicrobial activity, mainly attributed to the presence of phenolic compounds and flavonoids, capable of interfering with cell membrane integrity and the metabolic activity of microorganisms (Pobiega; Kraśniewska; Gniewosz, 2019). Chitosan, in turn, acts through electrostatic mechanisms, forming a physical and chemical barrier that hinders microbial growth (Mesgari; Aalami; Sahebkar, 2021). The combination of these agents, when used with thermal processing, proved effective in preserving product safety, reinforcing its applicability in formulations with a clean-label appeal.

Regarding sensory analysis, the absence of statistically significant differences between formulations for individual sensory attributes indicates that the incorporation of fish protein concentrate did not compromise the product's sensory acceptance. These findings indicate sensory equivalence between the control bread and the enriched formulations, even with the addition of an unconventional ingredient, which is desirable from a technological and consumer acceptance standpoint.

Although there was no statistical difference between the samples, the T30 formulation's high median indicates high absolute sensory acceptance. This behavior is common in tests conducted with untrained tasters, where the variability

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of individual responses can limit the detection of statistical differences, even when the products show high acceptance.

The acceptability index proved to be a more sensitive parameter for discriminating between the formulations, indicating lower acceptance of T15 and superior performance of T30, which was close to the control. This result suggests that greater inclusion of FPC favored overall product acceptance, reinforcing the formulation's sensory viability when enriched with 3% fish protein concentrate.

Additionally, the results of the acceptance and purchase intention tests indicate a greater predisposition among consumers towards the T30 formulation. The high acceptance observed may be associated with the evaluators' profiles, as individuals with greater interest in health, well-being, and nutritional quality tend to be more inclined to accept whole and enriched products. Studies indicate that consumer behavior significantly influences the sensory perception and intention to consume functional foods, especially those associated with nutritional and health benefits (Baker *et al*., 2022).

Overall, the results demonstrate that incorporating fish protein concentrate at a moderate level enables the development of a sensorially acceptable whole-wheat bread, with potential for market niche entry among physically active consumers or those with greater nutritional awareness.

The approximately 25% increase in crude protein content observed in formulation T30 confirms the efficacy of FPC as a functional ingredient for the fortification of bakery products. Similar results have been described in breads enriched with fish protein concentrates or hydrolysates, in which significant increases in protein content are obtained without relevant detriment to technological properties (Bastos *et al*., 2014; Desai *et al*., 2021). This increase represents a significant nutritional gain, especially given the widespread acceptance and high consumption of whole-wheat breads, which consolidate them as strategic matrices for protein enrichment of the diet.

The significant reduction in ash content observed in formulation T30 can be attributed to the removal of calcium propionate from the enriched formulation, since this additive constitutes a direct source of minerals. The maintenance of moisture, lipid, carbohydrate, and total energy value levels between formulations indicates

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that the partial replacement of wheat flour with FPC promoted a qualitative improvement in the nutritional composition, without compromising the energy balance or the physicochemical stability of the product, a desirable aspect from a technological and sensory point of view (Oprea *et al*., 2024).

Regarding technological parameters, the absence of significant differences in yield between the control bread and the T30 formulation indicates that the inclusion of 3% FPC did not affect mass losses during baking or the final product structure. Similar results were observed in studies with breads enriched with fish by-products at moderate levels of substitution (Monteiro *et al*., 2019). Water activity remained within the typical range for fresh breads, indicating that FPC did not significantly alter the water-protein-carbohydrate interaction in the product matrix (Centenaro *et al*., 2007).

The observed oxidative stability, evidenced by similar TBARS values between the formulations, indicates that the addition of the protein concentrate did not promote lipid peroxidation. This result is particularly relevant, as fish-derived ingredients are rich in polyunsaturated fatty acids, which are more prone to oxidation (Dragoev, 2024). The maintenance of low malondialdehyde levels suggests a protective effect of the formulation, possibly associated with the antioxidant action of propolis and chitosan, as described in previous studies (Khabbazi; Mansouripour; Saremnezhad, 2023).

Finally, the absence of significant changes in instrumental color parameters (L*, a*, and b*) confirms that the inclusion of FPC at a moderate level did not compromise the product's appearance. The observed color stability is consistent with the high sensory acceptance rates and with studies that report the maintenance of color in breads fortified with fish proteins at similar concentrations (Fagundes; Rocha; Salas-Mellado, 2018; Zebib *et al*., 2020).

5. CONCLUSION

Whole wheat bread enriched with 3% fish protein concentrate, combined with propolis and chitosan as natural preservatives, proved to be technologically viable, microbiologically safe, and sensorially acceptable. The enriched formulation

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improved the product′s nutritional value, with a significant increase in protein content, without compromising its physicochemical, technological, or oxidative stability.

Replacing calcium propionate with natural preservatives maintained the bread′s microbiological quality, highlighting the potential of propolis and chitosan as effective alternatives aligned with consumer demand for cleaner-label foods. Furthermore, the use of protein concentrate derived from by-products of the fishing industry reinforces formulation′s sustainability, contributing to the valorization of waste and the promotion of the circular economy.

Therefore, enriched whole wheat bread is a promising alternative for the baking industry, combining technological innovation, nutritional improvements, and sustainability. Future studies are recommended to evaluate the product's shelf life, storage stability, and economic viability on an industrial scale, to expand its commercial applicability.

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