Agroind. sci. 15(1): 7-18 (2025)

Agroindustrial Science

Website: http://revistas.unitru.edu.pe/index.php/agroindscience

Escuela de Ingeniería Agroindustrial

Universidad Nacional de Trujillo

Development of a fortifying grain similar to rice (*Oriza sativa*) enriched with anchovy peptides (*Engraulis ringens*)

Deyvis Espinoza Mendoza[1]; Augusto Castillo[2] *

[1] Escuela de Posgrado de la Universidad Nacional del Santa, Av. Universitaria s/n Urb. Bellamar, Nuevo Chimbote, Ancash. Perú.
[2] Departamento Académico de Agroindustria y Agronomía, Universidad Nacional del Santa; Av. Universitaria s/n Urb. Bellamar, Nuevo Chimbote, Ancash. Perú.

ORCID de los autores:
D. Espinoza Mendoza: https://orcid.org/0000-0002-2126-4035
A. Castillo: https://orcid.org/0000-0001-9237-8542

ABSTRACT

The aim of this study was to develop a rice-like fortifying grain enriched with anchovy (*Engraulis ringens*) peptides obtained through extrusion for use as a commercial rice fortifier. The study utilized arrocillo, a by-product of rice processing (*Oryza sativa*), mixed with anchovy peptides in powdered form obtained by enzymatic hydrolysis. The fortifying grain were optimized using response surface methodology to maximize protein content and degree of similarity to rice while minimizing mass loss during cooking. The optimal formulation consisted of 16.19% hydrolyzed anchovy protein concentrate, 0.19% additive (SIN 471), and 83.64% broken rice flour. The resulting fortifying grain demonstrated a protein content of 18.77%, a similarity score of 6.12 on a scale of 1 to 9 relative to rice, and a cooking mass loss of 8.22%. Industrial scale-up tests validated these results, demonstrating that the developed grain is an ideal rice fortifier due to its high biological value protein content, low cost compared to other animal protein sources, high similarity to rice, and acceptable cooking loss.

Keywords: Fortifying grain; fish protein extrusion, enzymatic hydrolysis.

1. Introduction

Peru ranks among the leading countries in hydrobiological product extraction, with anchovies *(Engraulis ringens*) being the predominant catch. In 2023, Peru landed a total of 3,495,003 metric tons (MT) of fish, of which 58.74% (2,053,073 MT) were anchovies. Only 3.52% (72,253 MT) allocated for direct human consumption divided into 38 MT for fresh consumption, 40,179 MT for canned production, 14,252 MT for frozen products, and 17,783 MT for cured production (PRODUCE, 2024). Frozen and cured anchovies and 20% of canned anchovy products are exported. Based on the current population of Peru, estimated at 34,039,000 inhabitants (Hilario et al., 2024), the per capita consumption of anchovies for direct human consumption is calculated to be 1.06 kg per annum.

Despite being rich in proteins, particularly lysine, polyunsaturated fatty acids (EPA and DHA), vitamins A and D, and minerals like potassium, iron, and phosphorus (León & Kung, 2021), which could potentially address nutritional deficiencies, the consumption of anchovies is limited by their distinctive and strong flavor (Majluf et al., 2017).

The wide availability of anchovy in Peru and its nutritional benefits offer significant potential for producing fish protein hydrolysates, which could serve to transform this species into an ingredient for daily consumption. This can be achieved by using commercial enzymes to hydrolyze proteins, which are currently being employed (Senadheera et al., 2021; Kakko et al., 2022; Valerio et al., 2023). Fish hydrolysates are valued for their nutritional properties, which stem from low molecular weight peptides formed during hydrolysis (Abuine et al.,

Recibido 6 noviembre 2024
Aceptado 4 enero 2025

* Autor correspondiente: acastillo@uns.edu.pe (A. Castillo)
DOI: http://doi.org/10.17268/agroind.sci.2025.01.01

2019). They have been used as food additives, supplements, or fortifiers to address human nutrition challenges (Honrado et al., 2023).

Studies have demonstrated the nutritional benefits of products enriched with fish hydrolysates (Rivero-Pino et al., 2020; Lima et al., 2021). Additionally, fish protein concentrates are a common source of lysine in cereal-based diets (Fatima et al., 2024). However, beyond nutritional benefits, other factors such as techno-functional and culinary characteristics, industrial scalability, cost, raw material availability, acceptability, and sustainability must be considered to enhance the value and acceptability of the final product.

These hydrolysates can sustainably fortify mass-consumption foods like rice, which has a high per capita consumption in Peru (70 kg per year) (OECD et al., 2023; MIDAGRI, 2021). Rice processing produces broken rice, often unused for direct consumption. This by-product can be transformed into flour enriched with anchovy-derived nutrients to address rice's nutrient deficiencies, exacerbated by processing losses (Mohidem et al., 2022). Alternatives have been validated to enhance the nutritional quality of rice and utilize by-products such as broken rice through extruded rice analogues or similar products incorporating nutrient-rich ingredients (Lee et al., 2022; Ganachari et al., 2022). These products mimic widely accepted foods, align with culinary traditions, and serve as effective vehicles for improving nutritional intake (Budijanto & Yuliana, 2015; Noviasari et al., 2017).

This study aimed to develop a fortified rice analogue enriched with anchovy peptides obtained via enzymatic hydrolysis, exhibiting acceptable physical and sensory qualities, including protein content, grain similarity, and cooking performance.

2. Methodology

2.1. Raw materials and imputs

For the enzymatic hydrolysis experiments, adult-stage Peruvian anchovy (*Engraulis ringens*), measuring over 12 cm, was sourced from artisanal fisheries in Chimbote. The fish were thoroughly washed and prepared for processing. The enzymes Corolase 8000 and Corolase 7089 (AB Enzymes® GmbH(R)), were employed (Figure1).

For the extrusion experiments, broken rice flour derived from the milling of *Oryza sativa L.* cultivar IR-43 (a by-product from the Santa Valley, Ancash, Peru) was mixed with hydrolyzed anchovy protein concentrate powder and the food additive E471, serving as an antifoam, emulsifier, glazing agent, and stabilizer (Figure1).

2.2. Hydrolysis

Hydrolysis experiments (Table 1) were performed in a 3L bioreactor (model Ez2-Control, Applikon) without pH adjustment, starting at pH 6.2 ± 0.2, with constant stirring at 200 rpm. The process was optimized to minimize insoluble solids and fat, targeting a value close to 8 °Brix—a reference previously established for obtaining peptides between 400 and 1000 Da.

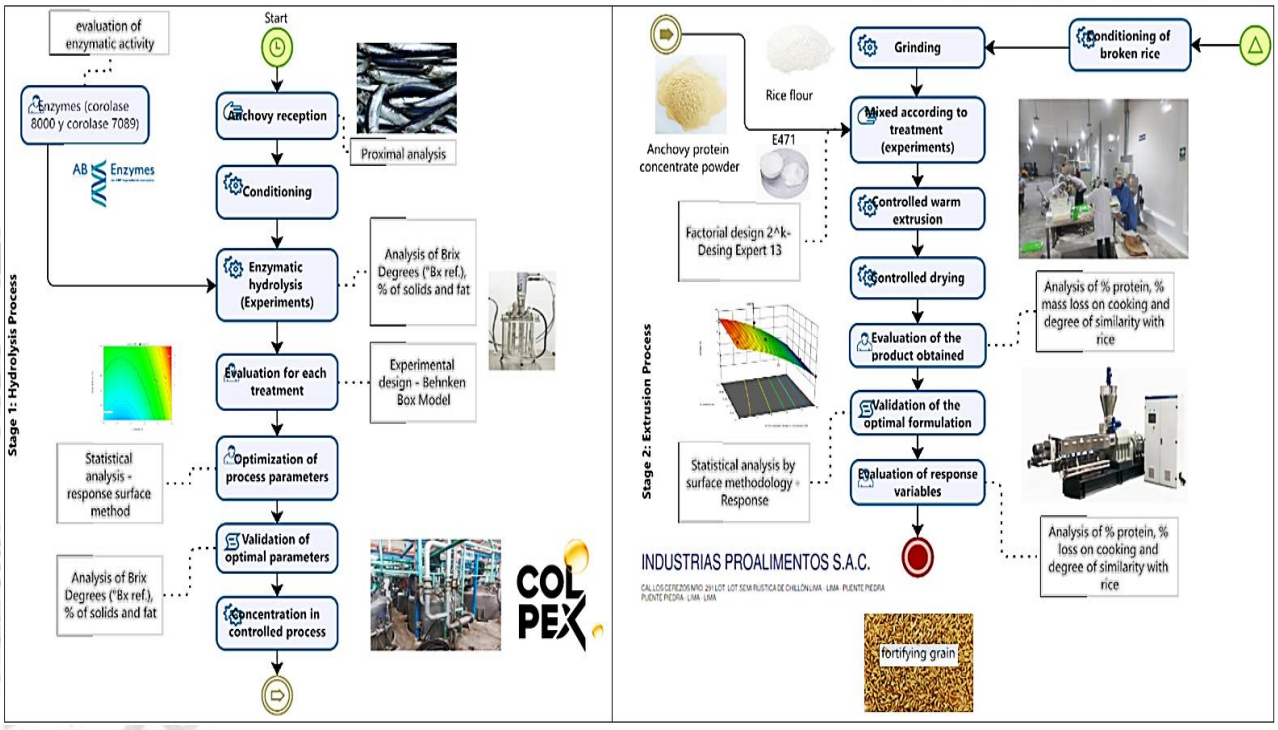

Figure 1. Development of a fortifying grain similar to rice grain enriched with Peruvian anchovy peptides.

Table 1
Factorial experimental design Box-Behnken model for experimental trials of enzymatic hydrolysis of Peruvian anchovy

Experiments	X1	X2	X3	Enzyme (%) ($\sum E_1, E_2$)	Enzyme (%)	Substratum (%)	Reaction time (min)
1	-1	-1	0	**0.2** (0.04+0.16)	0.2	0.45	45
2	0	0	0	**0.3** (0.06+0.24)	0.3	0.5	45
3	-1	1	0	**0.2** (0.04+0.16)	0.2	0.55	45
4	0	-1	1	**0.3** (0.06+0.24)	0.3	0.45	60
5	0	1	1	**0.3** (0.06+0.24)	0.3	0.55	60
6	1	0	-1	**0.4** (0.08+0.32)	0.4	0.5	30
7	0	1	-1	**0.3** (0.06+0.24)	0.3	0.55	30
8	1	1	0	**0.4** (0.08+0.32)	0.4	0.55	45
9	0	-1	-1	**0.3** (0.06+0.24)	0.3	0.45	30
10	1	-1	0	**0.4** (0.08+0.32)	0.4	0.45	45
11	-1	0	1	**0.2** (0.04+0.16)	0.2	0.5	60
12	1	0	1	**0.4** (0.08+0.32)	0.4	0.5	60
13	0	0	0	**0.3** (0.06+0.24)	0.3	0.5	45
14	0	0	0	**0.3** (0.06+0.24)	0.3	0.5	45
15	-1	0	-1	**0.2** (0.04+0.16)	0.2	0.5	30

After the designated reaction time, the mixture was filtered to remove spines and residues, and the enzymes were inactivated at 87 ± 5 °C. The samples were then centrifuged at 4000 rpm for 20 minutes. The resulting hydrolysate was concentrated via spray drying, following the process flow outlined in Figure 2.

2.3. Extrusion process

The extrudate formulation experiments (fortified grain), outlined in Table 2, were conducted using a twin-screw extruder (Model EDDT-200, Jinan Daton Machinery Co. Ltd.) equipped with rice-shaped die holes.

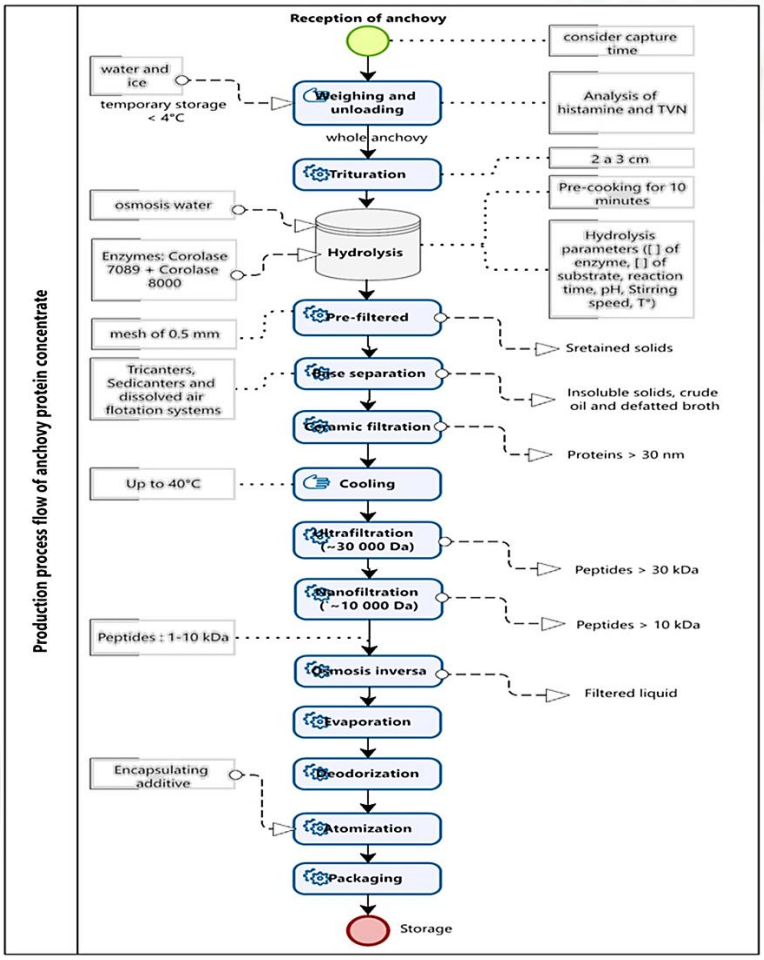

Figure 2. Process flow of anchovy protein concentrate production.

Table 2
2k factorial experimental design for the experimental trials of the fortifying grain formulation

Experiments	X_1	X_2	Whole anchovy hydrolyzed protein concentrate (%)	Additive (%)
1	1	1	30	0.2
2	0	-1	17.5	0.1
3	0	0	17.5	0.15
4	0	0	17.5	0.15
5	-1	0	5	0.15
6	-1	1	5	0.2
7	0	0	17.5	0.15
8	1	0	30	0.15
9	1	-1	30	0.1
10	-1	-1	5	0.1
11	0	1	17.5	0.2
12	0	0	17.5	0.15

A mixture of rice flour, hydrolyzed anchovy protein concentrate, and additive E471 was fed into the extruder with a moisture content of 25.4%. The process parameters were set based on conventional grain production methods. The optimal formulation was scaled up industrially following the parameters outlined in Figure 3.

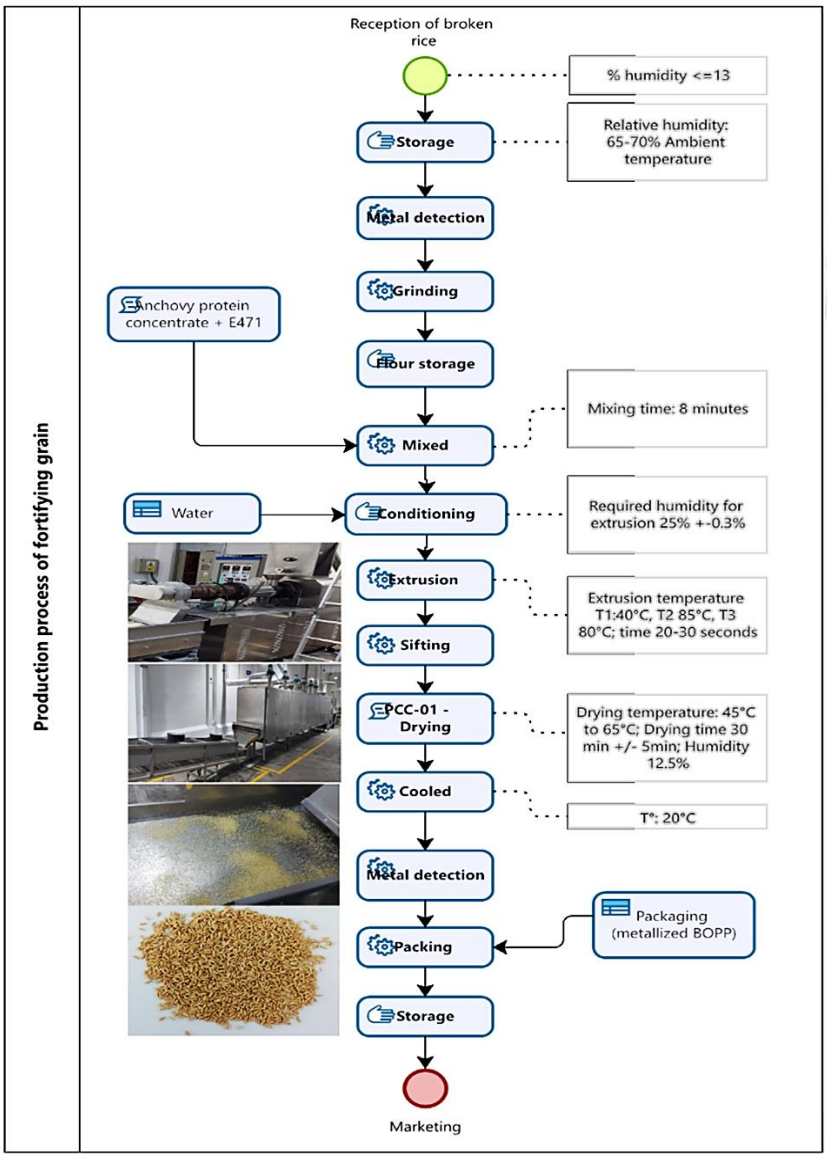

Figure 3. Process flow of fortifying grain production.

In order to determine the reference Brix degrees (ºBx ref.) of the hydrolysate, the methodology proposed by Roldán et al. (2021) was employed in a constant reaction volume (1 L) for each treatment. The equivalence of ºBx ref. with respect to the degree of hydrolysis was then determined in accordance with the procedure described by Nielsen et al. (2006) and Guo et al. (2019).

2.4. Analytical methods

To determine the enzymatic activity, the method established by Cupp-Enyard (2008) was employed, with modifications to simulate the pH conditions under which fish hydrolysis occurs (pH ~6.2). The tests were conducted with a 1:4000 (v/v) enzyme dilution for 10 min at temperatures of 50, 60, and 70 °C. The calibration curve was prepared with the amino acid L-tyrosine.

The insoluble solids in the hydrolysate were determined by centrifuging at 4000 rpm for 20 minutes, with the result expressed as a percentage of the total weight of the hydrolysate and the fraction of insoluble solids separated by centrifugation. To determine the percentage of insoluble solids (%SI), the following equation was employed: %SI = (Weight of the insoluble solids fraction)/(Total weight of hydrolysate) x 100.

The percentage of fat was determined by centrifugation at 4000 rpm for 20 minutes and was calculated based on the total weight of the hydrolysate and the fraction of fat separated by centrifugation. To ascertain the percentage of percentage of fat (%G), the following equation was employed: %G = (Fat fraction weight)/(Total weight of hydrolysate) x 100.

The proximate and nutritional evaluation of the hydrolysed protein concentrate powder was determined using the following methods: humidity (NTP-ISO 6496, 2022) and fat (NTP 204). The proximate and nutritional evaluation of the hydrolyzed protein concentrate powder was determined using the following methods: protein (NTP ISO 5983, 2018), ash (AOAC 942.05, 2019), digestibility (AOAC 971.09, 1999), histamine (NCh 2637, 2021), chlorides (AOAC 937.09, 2019), apparent density and compacted density (ISP, 2020), TBVN (IRAM 15025-2, 1985), particle size (NTP 204. The following parameters were analyzed: acidity (AOAC, 2012), microbiological (Enterobacteria, yeasts and molds (ICMSF, 2000), *Listeria monocytogenes* (Hitchins et al., 2022), and Salmonella (ISO 6579-1, 2020), heavy metals (Arsenic, cadmium, lead, mercury (AOAC, 2016), and fluorine (AOAC). Additionally, the analysis included the amino acid profile (ISO 13903:2005) and micronutrients (sodium, potassium, iron, phosphorus, magnesium, chromium, and molybdenum) (U.S. EPA, 1994).

The protein content of the fortifying grain was determined in accordance with the methodology established by INACAL (2018) in NTP 205.005:2018.

2.6. Statistical analysis

The data were subjected to an analysis of variance (ANOVA) ($p < 0.05$) using the Desing Expert® V. 13 statistical software. The Response Surface Method (RSM) was employed for the optimization of the parameters using the Composite Desirability Profile.

3. Results and discussion

3.1 Enzymatic hydrolysis of anchovy

The enzymatic activity of the commercial enzymes used on the substrate at a fixed process temperature (60 °C) and anchovy pH (6.2 ± 0.2) was measured at 1050.08 ± 110.2 U/mL for Corolase 7089 and 850.7 ± 115.1 U/mL for Corolase 8000. The temperature was chosen to standardize the industrial process and falls within the efficiency range for both enzymes. Corolase 7089 operates optimally at temperatures between 5.5 and 8.5 °C and pH levels from 5.5 to 8.5, while Corolase 8000 functions best at temperatures from 6.0 to 11.0 °C and pH levels between 6.0 and 11.0. The reaction temperature, close to the optimal for maximizing hydrolysis, has been associated with increased bitterness in anchovy enzymatic hydrolysis (Valerio et al., 2023). This observation suggests that a combination of enzymes is required for optimal results.

About the hydrolysis process, the findings of the experimental investigation are presented in Table 3. The °Brix reference range varied from 6.5 to 12.5, corresponding to a degree of hydrolysis of 16-32%. The degrees of hydrolysis obtained in this study were lower than those reported for fish skin, squid viscera, and sturgeon cartilage. Specifically, this study yielded values of 50.1 ± 1.1%, 42.08 ± 0.58%, and 34.23%, which are lower than those reported by You et al. (2010), Haotian et al. (2024), and Lin et al. (2024). However, the results were higher than those for fish skin and muscle reported by Rodríguez-Azizi et al. (2020) and Huai et al. (2024), which were below 19.08% and 17.4% to 19.2%, respectively. These discrepancies may be due to differences in enzymes, physicochemical variables, fish species, and proximate composition.

Table 3
Analysis of variance for Bx ref., %SI and %G

Experiments	Enzyme 1 (Corolase 7089)	Enzyme 2 (Corolase 8000)	Experimental variables			Response variables					
			∑ Enzyme X1	Substratum X2	Reaction time X3	°Bx ref.		% SI		% G	
						Observed	Planned*	Observed	Planned*	Observed	Planned*
1	0.04	0.16	0.2	0.45	45	7	7.13	31.62	31.55	2.4	2.23
2	0.06	0.24	0.3	0.5	45	7.5	8.29	34.16	35.61	2.8	2.8
3	0.04	0.16	0.2	0.55	45	8	8	33.62	33.55	2.6	2.65
4	0.06	0.24	0.3	0.45	60	9	9	33.96	33.51	2.6	2.64
5	0.06	0.24	0.3	0.55	60	10.5	11.13	44.11	43.53	2.7	2.51
6	0.08	0.32	0.4	0.5	30	8	8.13	39.47	38.89	2.8	2.66
7	0.06	0.24	0.3	0.55	30	10	9.38	40.35	4.93	2.4	2.36
8	0.08	0.32	0.4	0.55	45	12.5	12.5	38.09	38.16	2.8	2.98
9	0.06	0.24	0.3	0.45	30	7.5	7.25	30.47	30.92	1.4	1.59
10	0.08	0.32	0.4	0.45	45	9	9.12	31	31.07	2.8	2.75
11	0.04	0.16	0.2	0.5	60	9	8.41	38.83	39.42	2.7	2.84
12	0.08	0.32	0.4	0.5	60	10.5	9.91	34.81	35.26	2.7	2.71
13	0.06	0.24	0.3	0.5	45	8.5	8.29	37.5	35.61	2.8	2.8
14	0.06	0.24	0.3	0.5	45	8	8.29	35.16	35.61	2.8	2.8
15	0.04	0.16	0.2	0.5	30	6.5	6.66	31.04	30.6	1.7	1.69

*Obtained by the software.

The enzymes used are expected to directly affect the proteins in the raw material. However, other factors, such as the percentage of insoluble solids and fat, are also anticipated to vary. The production of insoluble solids can result from suboptimal physicochemical conditions in the hydrolysate (in the case of non-hydrolyzed proteins) or from components that the enzymes cannot act on (e.g., bones, insoluble fat, bone residues, connective tissue), which are typically removed during the process (Baez-Suarez et al., 2016). In this context, the percentage of insoluble solids reflects the efficiency of the interactions among the evaluated parameters. Additionally, reducing the fat content of the hydrolysate is important for extending the shelf life of the final product (Kristinsson & Rasco, 2000).

The regression equations of the response surface analysis to obtain 8 °Bx Ref, minimize % SI and % fat were:

$$°BrixRef. = -160,786 + 820 * Enzyme + 673,04 * Substratum + 0,058 * Reaction\ time -689,29 * Substratum^2 \quad [Eq.\ 1]$$

$$\%SI = +196,77 - 300,67 * Substratum - 0,1051 * Reaction\ time - 2,08 * Enzyme * Reaction\ time + 0,009 * Reaction\ time - 5470 * Enzyme^2 * Substratum \quad [Eq.\ 2]$$

$$\%Fat = -31,51 + 13,88 * Enzyme + 0,37 * Reaction\ time - 0,18 * Enzyme * Reaction\ time - 0,00156 * Reaction\ time^2 \quad [Eq.\ 3]$$

The analysis of variance showed statistically significant results ($p < 0.05$) for the proposed models. The coefficients of determination (R^2) exceeded 0.93, with values close to 1 indicating a strong correlation and a good model fit to the observed data (Saleem et al., 2020). These findings suggest that the proposed models explain 93%, 96%, and 93% (R^2) of the effects of the independent variables (enzyme, substrate, reaction time) and their interactions on the responses (Mohanty et al., 2021).

The optimal process variables for achieving an 8 °Bx reference were estimated to be an enzyme concentration of 0.364 g/100 g substrate (0.085 g Corolase 7089 and 0.275 g Corolase 8000), a substrate concentration of 0.45%, and a reaction time of 31.87 minutes, yielding a desirability index of 0.799 to minimize sodium ion (SI) and glucose (G) percentages. Validation of the optimization model provided predictive values of 7.99 °Bx, 32.65% SI, and 2.6% G, which were close to the observed values of 7.91 °Bx, 33.08% SI, and 2.32% G, indicating a high degree of similarity within a 95% confidence interval (CI). These results demonstrate the model's effectiveness in predicting behavior and optimizing performance based on the response variables in the enzymatic hydrolysis of Peruvian anchovy.

The hydrolysate obtained under these optimal conditions had a refractive index of 7.91 ± 0.30 °Brix, corresponding to a degree of hydrolysis of 14.03%. Higher degrees of hydrolysis have been

reported for the same species. Pandia et al. (2013) achieved 19% using Protamex with a 90-minute reaction. Roldán et al. (2021) reported 39% using whole anchovy with Protamex and Flavourzyme over 2 hours. Sifuentes-Penagos et al. (2018) achieved 6.90% in a 60-minute reaction. Valerio et al. (2023) reported 14.8% using Corolase 8000 and Corolase 7089 in sequential reactions. These variations suggest that the degree of hydrolysis is influenced by factors such as reaction time, enzyme type and concentration, substrate type, and target peptide size. Differences may stem from the emphasis in these studies on maximizing hydrolysis without prioritizing product quality or industrial-scale feasibility.

The proximate and nutritional composition of the Hydrolyzed Anchovy Protein Concentrate Powder is as shown in Table 4. The product's moisture content (4.16%) makes it hygroscopic, necessitating an efficient, airtight mixing process. The TBVN and histamine values are within the permissible range (less than 200 ppm) according to SANIPES (2016). These values exceed those of conventional flours, including high-quality Type A fishmeal (*Engraulis ringens*) with a minimum protein content of 68%, lysine of 5 g/100 g, and methionine of 1.3 g/100 g, as specified in NTP 204.035 (2023). Unlike conventional fishmeal, this protein concentrate is free from sand. Heavy metal levels are also within the maximum permissible limits set by Peru's health authority (SANIPES, 2016). Notably, the concentrate has high lysine (5.92 g/100 g) and threonine (2.59 g/100 g) contents, both of which are limiting amino acids in rice (Latham, 2002). The microbiological results comply with the maximum limits established in Peru's Health Standard for food and beverages for human consumption (MINSA, 2008). The product has a shelf life of 24 months when stored in an airtight metal container at room temperature.

The proximate composition of rice flour was found to be 10.9% moisture, 7.45% crude protein, 0.83% fat, and 0.61% ash, consistent with Wenhan et al. (2019). The moisture content falls within the permitted range (maximum 14%) for rice, as defined in NTP 205.011 (2023).

3.2 Optimize the fortifying grain formulation

To obtain the fortifying grain, we must consider the interactive effect of the experimental variables (percentage of protein concentrate in powder and additive) on the response variables (protein content (% P), mass loss during cooking (% PC), and degree of similarity (°S)). Table 5 shows the values obtained using the factorial design.

Based on the results obtained, the regression equations of the response surface analysis for %P, %PC and °S were:

$$\%P = 7{,}92 + 0{,}87 * \text{Hydrolyzed Protein Concentrate} - 17{,}68 * \text{Additive} - 0{,}24 * \text{Hydrolyzed Protein Concentrate} * \text{Additive} - 0{,}0034 * \text{Hydrolyzed Protein Concentrate}^2 + 53{,}50 * \text{Additive}^2 \quad \text{[Eq. 7]}$$

Table 4
Proximal and nutritional composition of anchovy protein concentrate

Proximal analysis and nutritional value	Reported values	Amino acid profile	Content	Mineral profile	Content
Humidity	4.16%	**Phenylalanine**	3.66 g/100g	**Heavy metals**	
Protein	81.41%	**Isoleucine**	2.25 g/100g	Arsenic	2.85 mg/kg
Fat	0.52%	**Leucine**	4.78 g/100g	Cadmium	0.025 mg/kg
Ashes	10.41%	**Lysine**	5.92 g/100g	Mercury	<0.01 mg/kg
Chlorides	4.18%	**Methionine + Cystine**	2.11 g/100g	Lead	<0.02 mg/kg
TCVN	98 mg/100g	**Threonine**	2.59 g/100g	**Micronutrients**	
Histamine	28.4 mg/100g	**Tryptophan**	0.50 g/100g	Sodium	9.136 g/kg
Digestibility to pepsin	98.04%	**Valine**	2.95 g/100g	Potassium	11.39 g/kg
Apparent density	0.20g/mL	Alanine	4.72 g/100g	Iron	59.61 mg/kg
Compact density	0.24g/mL	Arginine	3.76 g/100g	Phosphorus	5.126 g/kg
Particle size		Glycine	4.29 g/100g	Magnesium	948.8 mg/kg
Between 1.68 and 0.074 mm	88.82%	**Histidine**	3.50 g/100g	Chromium	2.39 mg/kg
Greater than 1.68 mm	0.00%	Aspartic acid	5.81 g/100g	Molybdenum	0.17 mg/kg
Less than 0.74 mm	11.18%	Glutamic acid	9.26 g/100g		
Acidity	0.07%	Proline	2.59 g/100g	Fluoride	4.22 mg/kg
		Serine	2.45 g/100g		
Microbial analysis	Enterobacteriaceae	Yeasts	Molds	Salmonella	
CFUs/g	<10	<10	<10	Not detected	

Table 5
Experimental results of the factorial model for the preparation of the rice analogue

Experiments	Experimental variables		Response Variables					
	Hydrolyzed protein concentrate (%)	Additive (%)	Protein (%P)		Loss of mass due to cooking (%PC)		Similarity to grain of rice (°S)	
			observed	Provided*	observed	Provided*	observed	Provided*
1	30	0.2	28.25	28.2	38.16	35.64	2.3	2.5
2	17.5	0.1	20.48	20.5	11.05	8.3	4.5	5.87
3	17.5	0.15	20.02	20.08	8.95	8.7	6.3	6.27
4	17.5	0.15	20.12	20.08	8.89	8.7	6.5	6.27
5	5	0.15	10.62	10.58	3.32	2.59	8.2	8.33
6	5	0.2	10.55	10.57	2.35	1.08	7.9	8.73
7	17.5	0.15	20.13	20.08	9	8.7	6.4	6.27
8	30	0.15	28.42	28.51	28.13	29.9	2.2	2.1
9	30	0.1	29.13	29.09	25.13	25.88	1.8	1.7
10	5	0.1	10.83	10.85	3.82	5.82	8.9	7.93
11	17.5	0.2	19.89	19.92	7.02	10.81	7.4	6.67
12	17.5	0.15	20.08	20.08	9.02	8.7	6.5	6.27

*Obtained by the software.

$$\%PC = 23{,}53 - 1{,}47 * \text{Hydrolyzed Protein Concentrate} + 5{,}80 * \text{Hydrolyzed Protein Concentrate} * \text{Additive} + 0{,}04 * \text{Hydrolyzed Protein Concentrate}^2 \quad \text{[Eq. 8]}$$

$$°S = 7{,}37 - 0{,}014 * \text{Hydrolyzed Protein Concentrate} + 8{,}00 * \text{Additive} - 0{,}0067 * \text{Hydrolyzed Protein Concentrate}^2 \quad \text{[Eq. 9]}$$

The analysis of variance confirmed the significance of the models, which were deemed adequate, as indicated by R^2 values exceeding 93% (Saleem et al., 2020). The optimal solution involves 16.19% hydrolyzed protein concentrate and 0.19% additive to maximize %P, minimize %PC, and maximize °S, achieving a desirability index of 0.65. Figure 4a illustrates that a higher %P requires a higher concentration of hydrolyzed protein concentrate. Similarly, %PC increases with higher concentrations of hydrolyzed protein concentrate (Figure 4b), while °S decreases (Figure 4c). The additive has no significant impact on the response variables, except for %PC, confirming its minimal influence in the analysis of variance. Food additives are employed for specific purposes such as enhancing shelf life, emulsifying, and improving texture (Saltmarsh, 2020). Added at minimal levels (Wu, 2021), they do not impact the proximate composition of the mixture and serve purely technological functions.

The protein content (%P) of the developed fortifying grain was 18.77%, exceeding the protein content of rice flour by approximately 168% (~7%). This demonstrates that the developed grain is suitable for use as a rice fortifier. Studies on extrudates enriched with hydrobiological proteins include Tapia et al. (2023), who reported protein levels between 11.2% and 15.39% when using squid flour, and Valenzuela-Lagarda et al. (2021), who noted that protein content in extrudates could exceed 40% of the proximate value. Industrially, protein concentrates from fish of the Engraulidae family are used in extruded snacks, such as keropok in Malaysia. Additionally, products such as cheese sticks with anchovy powder and tempeh sticks with anchovy have been developed (Kari et al., 2022).

The protein content of the developed fortifying grain surpasses that of commonly consumed foods, such as fresh cow cheese (17.5 g/100 g), whole evaporated milk (6.3 g/100 g), boiled chicken eggs (12.8 g/100 g), and chicken liver (18.0 g/100 g). It is comparable to whole anchovy (19.1 g/100 g), chicken breast (19.2 g/100 g), chicken meat (21.4 g/100 g), and beef (21.3 g/100 g) (Reyes-García et al., 2018). This indicates that anchovy-based products can enhance daily diets and help reduce malnutrition, as rice is a staple food accessible across socioeconomic levels.

The production cost of the fortifying grain was $2.60 per kg, which is lower than other protein sources available in Lima, Peru, such as pork ($4.74/kg), whole guinea pig ($7.89 for 600 g), turkey ($5/kg), duck ($4.47/kg), and beef ($5.26/kg), and comparable to raw chicken ($2.59/kg) (MIDAGRI, 2024).

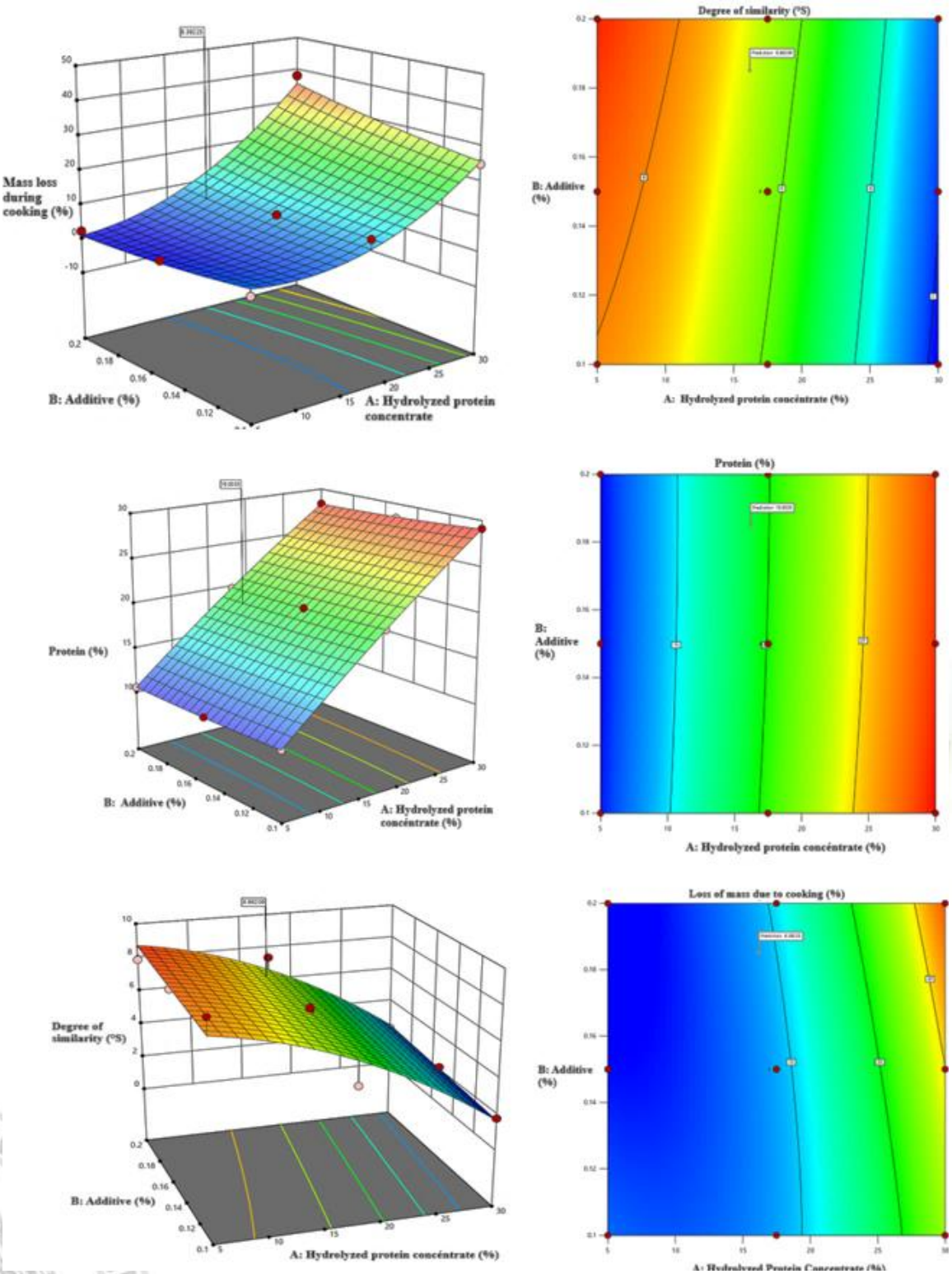

Figure 4. Response surface and contour plots for maximizing %P and °S, minimizing % PC; b) Substrate additive interaction for %P; c) Substrate additive interaction for %PC; Substrate additive interaction for °S.

3.3. Similarity to Commercial Rice Grain

The fortifying grain obtained from the validated formulation had a °S of 6.12 and average dimensions of 7 mm in length and 2 mm in thickness, classifying it as long grain according to NTP 205.011 (2023). The color was not evaluated, as it can vary based on processing methods and rice type. The similarity in characteristics indicates that the developed grain can be mixed with any rice variety (e.g., aged, parboiled, brown, or milled rice), including the type approved for rice fortification in Peru (Figure 5).

Figure 5. Degree of similarity of the developed fortifying grain (3) with respect to processed rice (2), brown rice (4) and fortifying grain approved for rice fortification in Peru (1).

3.4. Mass loss during cooking of the fortifying grain

The validated formulation exhibited a mass loss of 8.22% during cooking, which meets quality standards for cooking stability. Mass loss during cooking is a quality indicator in the assessment of partial wheat flour substitution, and it should not exceed 9% to maintain product quality (Hoseney, 1991).

4. Conclusions

The fortifying grain developed from broken rice flour enriched with anchovy peptides demonstrated a protein content of 18.77%, exceeding the protein content of commercial rice by 150.27%. It had a degree of similarity of 6.12 on a scale from 1 to 9 and an acceptable cooking mass loss of 8.22%. In addition, validation through pilot industrial scaling tests confirmed that this grain could be an ideal rice fortifier. It offers the benefits of including anchovy protein peptides, cost-effectiveness compared to other animal protein sources, high similarity to rice, and acceptable cooking stability.

This fortified grain can serve as an effective means for the comprehensive fortification of rice, incorporating both macronutrients (anchovy peptides) and micronutrients, potentially contributing to the reduction of anemia and chronic malnutrition rates in Peru and worldwide. However, additional analyses such as sensory evaluation, mechanical properties analysis and structural analysis (microscopy) must be performed to enable its mass production and subsequent commercialization.

Acknowledgements

Special thanks to Colpex International S.A.C. and Industrias Proalimentos S.A.C. for providing access to their laboratory and production facilities.

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