



Simplex-centroid mixture design as a tool to evaluate the effect of added flours for optimizing the formulation of native Brazilian freshwater fish burger

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ABSTRACT

The development of fish-based products can meet consumers' desires related to sensory, nutritional, and convenience aspects. In addition, it can contribute to the waste reduction of undervalued fish portions. This study presents the results of the optimization of fish burger formulations based on mechanically separated meat (MSM) of tambaqui (*Colossoma macropomum*) with oatmeal and cassava starch, using a simplex-centroid mixture design (SCMD) with total flour concentrations ranging from 0% to 10%, and MSM from 82.5% to 92.5%. The formulations were evaluated for physicochemical, yield, and sensory characteristics. The addition of flour had a positive effect ($p \leq 0.05$) on physicochemical and sensory properties, resulting in a 12% higher cooking yield compared to the formulation without flour. All formulations scored high acceptance; however, the formulation with the binary combination between flours showed the best ranking in the preference test and presented a score of 7.58 on the 9-Point hedonic Scale of global acceptance. Hardness was higher in the pure pseudo-component combinations but provided a reduction in binary and ternary combinations. The study shows the importance of optimization tools in product development aiming to consider yield and cost aspects.

1. Introduction

The nutritional importance of fish and its performance in reducing cardiovascular disease and positive effects on the anti-inflammatory and cognitive systems are known (Chen, Jayachandran, Bai, & Xu, 2022; Krittanawong et al., 2021; Martí & Fortique, 2019; Mohanty et al., 2019). Notwithstanding the recommendations for consumption, there is concern about the origin of fish, as some marine stocks are potentially depleted (WHO, 2003). Thus, approaches to increasing consumption based on aquaculture products are gaining momentum, as aquaculture techniques are seen as a window of opportunity for sustainable expansion (Gephart et al., 2021; Nature, 2021).

The study by Naylor et al. (2021) corroborates this context, indicating that the inclusion of sustainably managed fish in the human diet will depend on the species exploited and on production practices. Aquaculture has been developing exponentially with increasingly advanced systems, representing a solution for a continued supply of fish (FAO, 2020). Brazilian aquaculture follows this trend. In 2020

aquaculture production reached a record with an annual growth of 5.93% (Brazilian Association of Fish Farming, 2021). Nevertheless, productive challenges do persist, mainly related to two aspects: environmental risks, due to the impacts on the reduction of native ichthyofauna through the negligent control of exotic species (Fragoso-Moura, Porto, Maia-Barbosa, & Barbosa, 2016; Latini & Petrere, 2004; Pelicice, Vitule, Lima Jr., Orsi, & Agostinho, 2013); and the nutritional performance of each species (Berntsen et al., 2021; Henderson & Tocher, 1987).

In this context, native species with suitable productive performance tend to stand out, especially the tambaqui (*Colossoma macropomum*, Cuvier 1818), a native species of the Amazon River basin, currently among the most commercialized and widely spread native species in Brazilian fish farming, with remarkable productive and commercial records (Brazilian Association of Fish Farming, 2021). The production technology packages for the species are adapted to different cultivation systems, stocking densities, and varied feeding (Merola & Cantelmo, 1987; Reis et al., 2019; Santos et al., 2021).

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Although there is a remarkable overview in terms of production, the consumption of fish from aquaculture is restricted by the choice profile of consumers, who still choose species from extractive capture as they believe they are related to safety and trustworthiness (López-Mas et al., 2021; Mitra, Khatun, Prodhan, & Khan, 2021). A study carried out in different regions of Brazil showed a lack of consumer knowledge about the origin of fish; most consumers accepted Nile tilapia (*Oreochromis niloticus*) but rejected fish from aquaculture. Considering that the supply of Nile tilapia (*Oreochromis niloticus*) in Brazil is exclusive from aquaculture, there is a need to define strategies for cultural change in fish consumption (Pedroza Filho et al., 2020). Another study that evaluated the sensory acceptance of wild and cultivated tambaqui (*Colossoma macropomum*) indicated a difference in the organoleptic aspects, where cultivated fish has shown greater acceptance, probably due to the quality of possible management of aquaculture systems (Sousa et al., 2020).

Also, in the context of consumption, it is necessary to consider that fish is sold entirely or in traditional cuts, such as fillets, and processes that attempt to add value or use all parts of the fish are rare, resulting in the underutilization of food by the fish industry (FAO, 2020). An alternative at this point is the new food product development (NFPD), which can provide safe and nutritious food with the minimal addition of additives and based on renewable resources (Azanedo, Garcia-Garcia, Stone, & Rahimifard, 2020; Garcia-Garcia, Azanedo, & Rahimifard, 2021). Convenience food products are a possible strategy, being increasingly present in the choice of consumers who, in addition to convenience, value sensory and nutritional characteristics (Contini, Boncinelli, Gerini, Scozzafava, & Casini, 2018; Darian & Cohen, 1995). Although studies on fish-based convenience foods such as tambaqui (*Colossoma macropomum*) fish burgers indicate sensory acceptance (Anjos et al., 2021; Lima et al., 2020) there is still a need for research to improve the development process, focusing on commercial and healthiness aspects (Duran et al., 2017). Process approaches, such as cost reduction and fiber addition by applying flours and starches to fish burgers, have been discussed as in the study by Dilucia, Lacivita, Del Nobile, and Conte (2021). However, the effects of these ingredients were not evidenced. In another more detailed approach, the addition of flaxseed flour had a significant effect on moisture retention and cooking yield, and additions greater than 10% tended to lower consumer acceptance (Duman, 2020).

Applied statistical methods, such as optimization models, contribute to the formulation of new products, suitable for commercial scale, considering consumers' demands and preferences. Simplex-centroid mixture design (SCMD) results allow to identify the synergistic effects of mixtures and predict models that provide answers, such as high quality and low costs (Calado & Montgomery, 2003; Cornell, 2011).

The objective of this work was to develop a fish burger based on mechanically separated meat (MSM) obtained from tambaqui (*Colossoma macropomum*) to promote the consumption of Brazilian native fish from aquaculture production, with the addition of flour as a functional ingredient, using a simple design with three variables (MSM, oatmeal and cassava starch) to optimize a new clean label product formulation.

2. Material and methods

2.1. Raw materials and MSM preparation

Twelve samples (N = 12) consisting of tambaqui (*Colossoma macropomum*) bands were supplied by a local producer (Piracicaba/SP, Brazil). The samples, from the same batch and produced in an aquaculture system, were received frozen by the Fish Processing Laboratory ESALQ/USP (Piracicaba/SP, Brazil) and had an average weight of 1.2 kg. Then the bands were de-frosted, and the head and skin were removed before obtaining mechanically separated meat in a mincer (High Tech 250C). The mechanically separated meat (MSM) was homogenized and packed into 1 kg packages, frozen at -18°C . Other ingredients: oatmeal;

cassava starch; NaCl; monosodium glutamate, and spices, were purchased at the local market. The yields concerning the band and the MSM mass were registered and calculated according to the following equation.

$$\% \text{Skin yield} = \frac{\text{skin weight}}{\text{fish band weight}} \times 100 \quad (1)$$

$$\% \text{Head yield} = \frac{\text{head weight}}{\text{fish band weight}} \times 100 \quad (2)$$

$$\% \text{MSM yield} = \frac{\text{MSM weight}}{\text{fish band weight}} \times 100 \quad (3)$$

2.2. Formulation development of fish burgers using simplex-centroid mixture design

To optimize the fish burger formulation, the simplex-centroid mixture design (SCMD) method was applied using the Statistica® 13.3 software from TIBCO (Palo Alto, California, USA). The adjustment for the simplex region established for the study was delimited into three pseudo-components (independent variables (X1: MSM; X2: oatmeal; X3: cassava starch)), ranging from 0 to 1. The SCMD consisted of 7 different formulations, to avoid systematic errors, the experiments were performed randomly. Points 1, 2, and 3 (triangular vertices) correspond to the pure pseudo-components. Points 4, 5, and 6 were the binary mixtures of two, and point 7 (the center of the triangle) was the ternary mixture. The formulations are described as F1:0C0, F2:O10, F3:C10, F4:O5, F5:C5, F6:O5C5, F7:O3C3, respectively.

The design points in the Simplex-Centroid are represented in triangular diagrams by polynomial equations that will be defined by the q th-order mixture. In the case of ternary mixtures where $q = 3$ the special cubic model (Eq. (4)) must be used, allowing the prediction of the values of response surfaces.

$$\hat{y} = \sum_{i=1}^q \beta_i x_i + \sum_{i < j} \beta_{ij} x_i x_j + \sum_{i < j < k} \beta_{ijk} x_i x_j x_k + \varepsilon \quad (4)$$

Interactions are evaluated in b_i , b_{ij} and b_{ijk} ; x_i , x_j and x_k are the studied factors and \hat{y} is the experimental response to the factors.

Subsequently, the SCMD coded experimental points were configured in the original components (Table 1), the minimum and maximum levels of the independent variables, and the other ingredients were fixed in the formulations: NaCl (1.5%); monosodium glutamate (0.2%), white pepper (0.1%), garlic powder (0.3%), onion powder (0.4%) and ice (5%), were based on previous studies (Delfino et al., 2021; Oliveira et al., 2020) and pre-tests in the laboratory. Afterward, the formulations were weighed, and the ingredients were homogenized. Portions of 90g were molded into suitable formats of 10 cm in diameter, placed in low-density polyethylene bags, and stored at -18°C until processing and analysis.

Table 1
Simplex-centroid mixture design applied to optimize fish burgers formulation.

Run	Pseudo-components			Original Components (%)		
	X ₁	X ₂	X ₃	MSM (X ₁)	Oatmeal (X ₂)	Cassava Starch (X ₃)
1	1	0	0	92.5	0	0
2	0	1	0	82.5	10	0
3	0	0	1	82.5	0	10
4	0.5	0.5	0	87.5	5	0
5	0.5	0	0.5	87.5	0	5
6	0	0.5	0.5	82.5	5	5
7	0.333	0.333	0.333	85.84	3.33	3.33

2.3. Physicochemical and microbiological analysis of MSM and fish burgers

2.3.1. Microbiological analysis

To determine microbiological safety, 25 g of MSM and each formulation were analyzed in triplicate. Samples were homogenized (ITR MC-1204) with 225 mL of 0.1% peptone salt solution for enumeration of total coliforms, *Escherichia coli*, and coagulase-positive staphylococci, another 25 g sample was homogenized in 225 mL of 1% buffered peptone solution (pre-enrichment) for analysis of *Salmonella* spp.

The analyses of total coliforms and *Escherichia coli* were carried out following the AOAC Method 998.08:2002 (3M Petrifilm), coagulase-positive staphylococci according to ISO 6888-2:2021, both results were expressed in CFU/g⁻¹, and *Salmonella* spp. according to ISO 6579:2017, expressed as absence or presence per 25 g.

2.3.2. Proximate composition

For analysis of the proximate composition of MSM and fish burger formulations, the tests were performed on uncooked samples by AOAC official methods (2005). Crude protein was determined by the Kjeldahl method (using N x 6.25 as a conversion factor); the fat was extracted

$$\% \text{Diameter reduction} = \frac{\text{raw fish burger diameter} - \text{cooked fish burger diameter}}{\text{Raw fish burger diameter}} \times 100 \quad (6)$$

$$\% \text{Thickness reduction} = \frac{\text{raw fish burger thickness} - \text{cooked fish burger thickness}}{\text{raw fish burger thickness}} \times 100 \quad (7)$$

with hexane in a Soxhlet type extractor; humidity was determined by the gravimetric method in an oven with air circulation at 105 °C; the ash content was determined by incineration in a muffle at 550 °C. The carbohydrate level of the fish burgers was determined by subtracting the moisture, protein, fat, and ash percentages from 100. The energy value was determined based on 100 g, by multiplying the crude protein and carbohydrates by 4 and fat by 9.

2.3.3. Water activity (a_w) and pH measurement

Water activity (a_w) was determined using a standard hygrometer (AquaLab 4 TE, Decagon Devices, Pullman, USA). The pH was measured on the uncooked fish burger using a pH meter (Tecnal Tec-3MP, Piracicaba, Sao Paulo, Brazil). Measurements were performed in triplicate.

2.4. Fish burger evaluation analysis

The fish-burger samples were cooked on an electric plate at 150 °C for 4 min on each side until the internal temperature reached 75 °C, and then samples were cooled to room temperature (the same process was used in preparing samples for sensory analysis).

2.4.1. Instrumental color measurement

Color measurement of fish burgers was determined using a colorimeter (Chroma Meter CR-400, Konica Minolta, Japan), measuring an area of 8 mm in diameter. L^* (luminance ranging from 0 (black) to 100 (white)), color coordinates a^* (green to red), and b^* (blue to yellow) were examined. The analysis was performed in triplicate on each side of the fish burger, using three of each formulation, totaling 18 readings per treatment.

2.4.2. Texture profile analysis (TPA)

Texture profile analysis (TPA) was performed using a Texture Analyzer (Stable Micro Systems, TA-XT-PLUS, Godalming, Surrey, UK).

Cylindrical samples were cut from fish burgers, with a 17 mm diameter and a height of 10 mm, and subjected to a two-cycle compression test. Samples were compressed to 50% of their original height with a cylindrical aluminum probe (P/35 35 mm diameter), with a test speed of 1 mm s⁻¹ and a post-test speed of 10 mm s⁻¹. The analysis was performed using three fish burgers of each formulation, totaling nine values per treatment. The parameters determined were hardness, cohesiveness, springiness, and chewiness, as described by Bourne, Kenny, and Barnard (1978).

2.4.3. Technological cooking properties

The weight of the fish burgers was measured using an analytical scale and a digital caliper before and after the cooking process on three fish burgers of each formulation. Cooking yield, diameter reduction, thickness reduction, moisture retention, and fat retention were determined as described by Sánchez-Zapata et al. (2010) measured according to the following equations:

$$\% \text{Cooking yield} = \frac{\text{cooked weight}}{\text{raw weight}} \times 100 \quad (5)$$

$$\% \text{Moisture retention} = \frac{\text{cooked weight} \times \% \text{moisture in cooked fish burger}}{\text{raw weight} \times \% \text{moisture in raw fish burger}} \times 100 \quad (8)$$

$$\% \text{Fat retention} = \frac{\text{cooked weight} \times \% \text{fat in cooked fish burger}}{\text{raw weight} \times \% \text{fat in raw fish burger}} \times 100 \quad (9)$$

Moisture, fat (Soxhlet), and protein (Kjeldahl) of cooked fish burgers from Equations (7) and (8) were determined using AOAC (2005) methods.

2.5. Quantitative descriptive analysis

The quantitative descriptive analysis (QDA) was performed based on the recommendations of Stone and Sidel (2004). All procedures were approved by the Committee for Ethics in Research on Human Being (CEP ESALQ-USP) under number CAAE: 43321621.7.0000.5395. Candidates were recruited among students of the Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ-USP) through an online form. Nineteen candidates, out of the twenty-seven recruited, were pre-selected through a basic taste recognition test (no errors for 6 solutions) and an odor recognition test (a minimum of 8 correct answers out of a total of 10 solutions) according to ISO 3972:2011 and ISO 8586:2012.

2.5.1. Development of descriptive terminology

The sensory attributes were raised by the nineteen panelists based on a study by Quadros, Rocha, Ferreira, and Bolini (2015). After discussions to reach a consensus, the descriptive terms that were most important to characterize the appearance, aroma, taste, and texture were selected.

Table 2

Attributes, definitions, and reference samples developed by the sensory panel for MSM-based fish burgers obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Attribute	Descriptor	Definition	Reference
Appearance	Grilled color	Caramelized color intensity	Weak: fish burger baked in aluminum foil Strong: grilled fish burger
	Thickness	Visual perception of the characteristic thickness	Weak: fish burger with 0.5 cm thickness Strong: fish burger with 1.2 cm thickness
	Compaction	Perception of the completeness of the fish burger	Weak: minced meat fish burger Strong: fish burger based on MSM
Aroma	Fish odor	Intensity of characteristic fish odor	Weak: grilled fish burger Strong: fish burger cooked in water
	Rancid odor	Intensity of characteristic rancid odor	Weak: grilled fish burger Strong: fish burger dipped in soy oil
Flavor	Fish flavor	Perception of the amount of characteristic fish taste	Weak: grilled fish burger Strong: fish burger cooked in water
	Seasoned	Perception of the amount of seasoning and spices	Weak: grilled fish burger Strong: fish burger dipped in soy oil
	Saltiness	Perception of the amount of salt	Weak: fish burger control with low addition of salt and condiments Strong: grilled fish burger
	Greasy	Perception of the amount of fat	Weak: fish burger control with low addition of salt and condiments Strong: grilled fish burger
Texture	Hardness	Force required to deform the fish burger	Weak: fish burger grilled in 70 °C Strong: fish burger grilled in 150 °C
	Succulence	Perception of the amount of liquid released from the fish burger in the mouth	Weak: fish burger grilled in 70 °C Strong: fish burger grilled in 150 °C
	Crust	Perception of the crunchiness of the crust when chewing the fish burger	Weak: fish burger grilled in 70 °C Strong: fish burger grilled in 150 °C

The sensory panel, in consensus with the assistance of a leader, also defined the minimum and maximum intensity references for each attribute (Table 2).

2.5.2. Training and selection

Training for sensory memory formation and leveling was carried out in three sessions for each panelist, through direct contact with the maximum and minimum intensity references for each attribute. After the training stage, the panelists were selected according to their discriminatory power, repeatability, and consensus with the panel, according to ISO 8586:2012. Twelve out of nineteen were selected to perform analysis on the sensory profile of fish burgers. The final panel was composed of 60% women and 40% men, aged between 22 and 43 years.

2.5.3. Final assessment

Analysis was performed at the Sensory Analysis Laboratory of the Department of Agri-food Industry, Food and Nutrition (ESALQ-USP) in individual cabins (22 °C), under white light, according to ISO 8589:2007 and ASTM International (2017). The seven fish burger formulations were presented to the selected panelists on plates encoded with random three-digit numbers. The fish burgers were presented randomly and evaluated on an unstructured linear intensity scale of 90 mm in length for each attribute, using vocabulary developed by the trained panel, they were instructed to use water and a cream cracker to clean the palate between samples.

2.6. Sensory affective testing

The affective test was carried out with 12 trained panelists to verify the global acceptance of the fish burger as described by Stone and Sidel (2004). The test consisted of a 9-point hedonic scale with verbal anchors (1 dislike extremely and 9 like extremely), purchase intention (1 definitely will not buy and 5 definitely will buy), and a preference ranking test. The seven fish burger formulations were presented to trained panelists on plates encoded with random three-digit numbers.

2.7. Statistical analysis

All determinations were run at least three times. The values of different parameters were expressed as the mean standard deviation, using analysis of variance (ANOVA) and then Tukey's test was applied to compare the means with a significance level of 5%. Data were analyzed using the Statistica® 13.3 software from TIBCO (Palo Alto, California, USA).

3. Results and discussion

3.1. Physicochemical analysis and microbiological analysis

The yield of mechanically separated meat (MSM) of tambaqui (*Colossoma macropomum*) was 59.81% compared to the weight of the entire band. Considering the disposal of the head (24.92%) and skin (9.38%), the yield was 91.04%. Similar results were found in the MSM yield evaluation of catfish (*Clarias gariepinus*), with 57.33% and 93.88% in relation to living weight and clean trunk, respectively (Daga et al., 2020). The moisture content of the MSM was $71.89 \pm 0.24\%$, $10.05 \pm 0.17\%$ fat, $15.78 \pm 0.55\%$ protein, $1.28 \pm 0.04\%$, and $1.01 \pm 0.26\%$ ash and carbohydrate, respectively. Water activity was 0.99 and pH 6.53. Similar values were observed for the MSM of red porgy (*Pagrus pagrus*) (Guimarães et al., 2018), and MSM of catfish (*Brachyplatystoma vailantii*) (Oliveira, Lourenço, Sousa, Peixoto Joele, & Ribeiro, 2015).

The results of the proximate composition of fish burger formulations described in Table 3, showed significant differences for moisture, ash, fat, and protein contents ($p \leq 0.05$). As the raw material and ingredients used were the same, the variation in the final composition of the formulations was due to the interaction between the pseudo-components. However, the addition of flours had no significant effect ($p \geq 0.05$) on a_w and pH, the results varied between 0.97 and 0.98, 6.40 and 6.47, respectively.

The main proximate constituent being moisture, it is also the component that suffers the most variation, mainly due to its interaction with fat, corroborating the most notable effect of the addition of flour on moisture and lipid composition. A study that evaluated the addition of cassava starch in the development of fish burgers also showed a reduction in moisture (Pires et al., 2017). Another study that evaluated the addition of oatmeal showed a reduction in moisture, and reduction in lipids in the formulation of fish burgers with Nile tilapia (*Oreochromis niloticus*) pulp (Braga, Pasquetti, Bueno, & Merengoni, 2008). However, the formulation with the highest concentration of oatmeal (F2:O10) had an additive effect on lipid concentration. A similar result was observed

Table 3Physicochemical composition of MSM-based fish burgers obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Form.	Pseudo-components			Responses							
	X ₁	X ₂	X ₃	Moisture	Ash	Fat	Protein	Carbohydrate	Kcal	a _w	pH
F1:O0C0	1	0	0	71.55 ± 0.31 ^a	2.67 ± 0.00 ^{ab}	8.82 ± 0.03 ^b	15.26 ± 0.63 ^{ab}	1.69	166.23	0.97 ^a	6.46 ^a
F2:O10	0	1	0	62.87 ± 0.40 ^c	2.83 ± 0.05 ^a	9.29 ± 0.02 ^a	16.21 ± 0.34 ^a	8.80	164.41	0.98 ^a	6.40 ^a
F3:C10	0	0	1	65.25 ± 0.37 ^d	2.61 ± 0.04 ^b	7.96 ± 0.04 ^e	14.37 ± 0.03 ^b	9.81	162.95	0.97 ^a	6.46 ^a
F4:O5	0.5	0.5	0	68.51 ± 0.47 ^{bc}	2.73 ± 0.10 ^{ab}	8.59 ± 0.12 ^c	15.85 ± 0.17 ^a	4.33	164.02	0.98 ^a	6.43 ^a
F5:C5	0.5	0	0.5	67.77 ± 0.07 ^c	2.72 ± 0.01 ^{ab}	8.82 ± 0.07 ^b	15.45 ± 0.06 ^{ab}	5.25	159.63	0.98 ^a	6.46 ^a
F6:O5C5	0	0.5	0.5	65.68 ± 0.20 ^d	2.41 ± 0.02 ^c	8.31 ± 0.05 ^d	15.09 ± 0.27 ^{ab}	8.51	168.41	0.98 ^a	6.44 ^a
F7:O3C3	0.333	0.333	0.333	67.56 ± 0.24 ^c	2.55 ± 0.03 ^{bc}	7.99 ± 0.02 ^e	15.08 ± 0.27 ^{ab}	6.82	162.42	0.98 ^a	6.47 ^a

Results are means ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

for the lipid composition in addition to more than 10% flaxseed flour in the fish burger (Duman, 2020).

The results on the proximate composition of the formulations showed values correlated with studies without the addition of fat substitutes, as in the study with fish burgers of silver catfish (*Rhamdia quelen*) filleting residue, which presented values of 67.89%, 2.97%, 7.24% and 16.74% for moisture, protein, lipid, ash, and carbohydrate content, respectively, when the addition of residues was greater (Bochi, Weber, Ribeiro, Victório, & Emanuelli, 2008). Anjos et al. (2020) found correlated values for moisture in tambaqui fish burgers (*Colossoma macropomum*), enriched with green banana and chitosan biomass. The flour concentrations used by the experimental design did not have a significant effect on the energetic value when compared to the formulation without flour.

To ensure the hygienic-sanitary quality of the study, the MSM and fish burger formulations were analyzed and presented following the established criteria by Normative Instruction No. 60 of the National Health Surveillance Agency (ANVISA) (BRAZIL, 2019). The results indicated the absence of *Salmonella* spp. (in 25g) and *Escherichia coli* (CFU/g) in all samples, and the samples of MSM, F2:O10, F4:O5, F5:C5, and F7:O3C3 presented values $\leq 1.0 \times 10^1$ for coagulase-positive staphylococci (CFU/g) and were absent in the other formulations. Microbiological patterns of the fish burger were also evidenced accordingly in the studies by Fogaça, Otani, Portella, Santos-Filho, and Sant'Ana (2015), in the microbiological evaluation of fish burgers with MSM of Nile tilapia (*Oreochromis niloticus*), and in the evaluation of the effects of grape pomace flour on quality parameters of salmon burgers (*Salmo salar*) (Cilli et al., 2019).

3.2. Texture profile

The use of trans fats in conventional products has been increasingly questioned by consumers. Conversely, it has a strong effect on the texture of molded animal foods (Hygreeva, Pandeia, & Radhakrishna, 2014). However, studies show that replacing functional ingredients like fibers and starches improves the stability of animal products (Schmiele, Mascarenhas, Barretto, & Pollonio, 2015; Talukder, 2015).

The results on the effect of adding flour on the fish burger texture are

Table 4Instrumental texture and color parameters (L^* , a^* and b^*) of MSM-based fish burgers obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Form.	Pseudo-components			Responses						
	X ₁	X ₂	X ₃	Hardness (N)	Cohesiveness	Springiness (cm)	Chewiness (N-cm)	L^*	a^*	b^*
F1:OOC0	1	0	0	20.46 ± 2.54 ^b	0.59 ± 0.04 ^a	0.66 ± 0.00 ^c	7.95 ± 0.49 ^b	56.04 ± 3.24 ^a	5.34 ± 1.07 ^a	18.94 ± 2.81 ^a
F2:O10	0	1	0	34.09 ± 4.44 ^a	0.61 ± 0.02 ^a	0.66 ± 0.03 ^c	13.82 ± 2.46 ^a	51.97 ± 2.20 ^a	7.13 ± 1.71 ^a	24.88 ± 3.00 ^a
F3:C10	0	0	1	38.67 ± 1.89 ^a	0.61 ± 0.03 ^a	0.79 ± 0.00 ^a	18.80 ± 1.74 ^a	54.59 ± 2.67 ^a	5.35 ± 1.34 ^a	21.25 ± 4.33 ^a
F4:O5	0.5	0.5	0	30.61 ± 2.78 ^a	0.54 ± 0.01 ^a	0.74 ± 0.02 ^{ab}	12.49 ± 1.62 ^a	52.21 ± 2.45 ^a	7.38 ± 2.05 ^a	24.64 ± 3.74 ^a
F5:C5	0.5	0	0.5	31.30 ± 6.93 ^a	0.57 ± 0.03 ^a	0.78 ± 0.01 ^a	14.00 ± 3.95 ^a	51.25 ± 2.25 ^a	7.17 ± 1.79 ^a	24.16 ± 3.73 ^a
F6:O5C5	0	0.5	0.5	24.21 ± 2.23 ^{ab}	0.55 ± 0.01 ^a	0.70 ± 0.01 ^{bc}	9.39 ± 0.93 ^{ab}	54.50 ± 2.61 ^a	5.93 ± 1.84 ^a	22.94 ± 3.83 ^a
F7:O3C3	0.333	0.333	0.333	24.19 ± 1.37 ^{ab}	0.52 ± 0.04 ^a	0.74 ± 0.03 ^{ab}	9.64 ± 0.88 ^{ab}	50.01 ± 1.94 ^a	8.93 ± 1.70 ^a	27.83 ± 3.51 ^a

Results are means ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

shown in Table 4. The hardness that describes the force required for deformation of the fish burger between the molar teeth showed similar values in the study with a fish burger of Nile tilapia (*Oreochromis niloticus*) (Bainy, Bertan, Corazza, & Lenzi, 2015).

According to Gao, Zhang, and Zhou (2014), there is an inversely proportional relationship between moisture and hardness, which explains the lower textural values of the formulation without the addition of flour (F1:OOC0) While cassava starch had an effect on textural characteristics, these effects were not visible on the response surface (Fig. 1).

The addition of wheat bran in biquara fish burgers (*Haemulon plumieri*) showed a reducing effect on hardness and cohesiveness (Raúl et al., 2018). However, the results of the addition of oatmeal and cassava starch in higher concentrations indicated an increase in hardness and cohesiveness, possibly because these ingredients are fibrous and tend to weaken myofibrillar bonds. It is necessary to consider that the addition of flour favors the retention of macronutrients and tends to improve the integrity of the product.

A previous study on the effects of starch properties on the textural characteristics of fish burgers, using surimi (*Merluccius hubsi*), showed similar results for cohesiveness and springiness, but hardness and chewiness values were lower (Coelho, Weschenfelder, Meinert, Amboni, & Beirão, 2007). Compared to beef burgers, the values of cohesiveness, springiness, and chewiness were also similar (Carvalho, Milani, Trinca, Nagai, & Barretto, 2017; Trevisan, Bis, Henck, & Barretto, 2016).

3.3. Color instrumental measurements

The results of the color parameters of the cooked fish burger formulations are shown in Table 4. Color assessment is an important tool for food products as it directly affects the sensory acceptance of consumers (Clydesdale, 1991) mainly for products that use cooking processes, such as fish burgers, which undergo various reactions such as the Maillard effect, which, in addition to adding a caramelized appearance, can mask undesirable colors (Ames, 1992; Starowicz & Zieliński, 2019). Similar results for L^* , a^* , and b^* values were found for the blended fish burger with different percentages of shrimp, camel, and ostrich (Shekarabi, Monjezi, Shaviklo, & Mohamed, 2020).

(Ames, 1992; Starowicz & Zieliński, 2019). Similar results for L^* , a^* ,

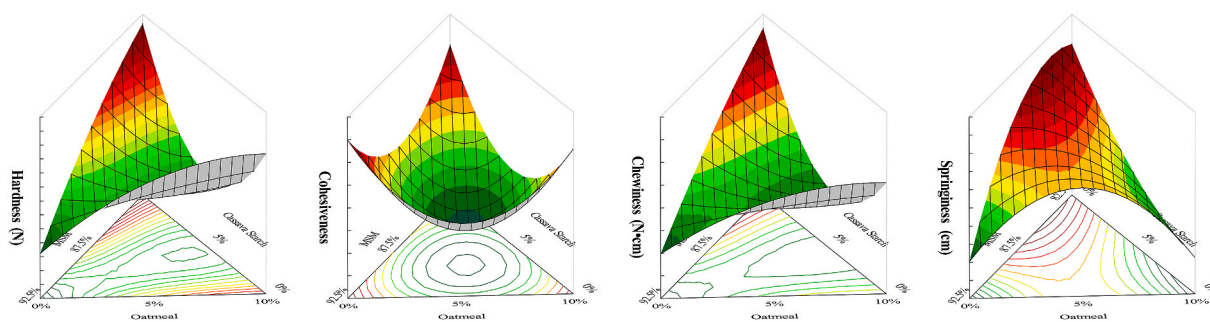


Fig. 1. Response surface plots for effects of adding oatmeal and cassava starch on the instrumental texture of fish burger formulations.

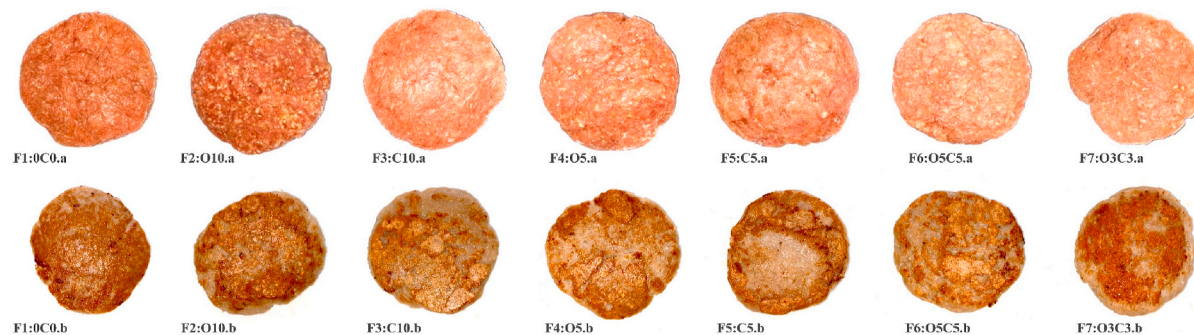


Fig. 2. Fish burgers with different concentrations of oatmeal and cassava starch. Line a: raw fish burger, line b: grilled fish burger.

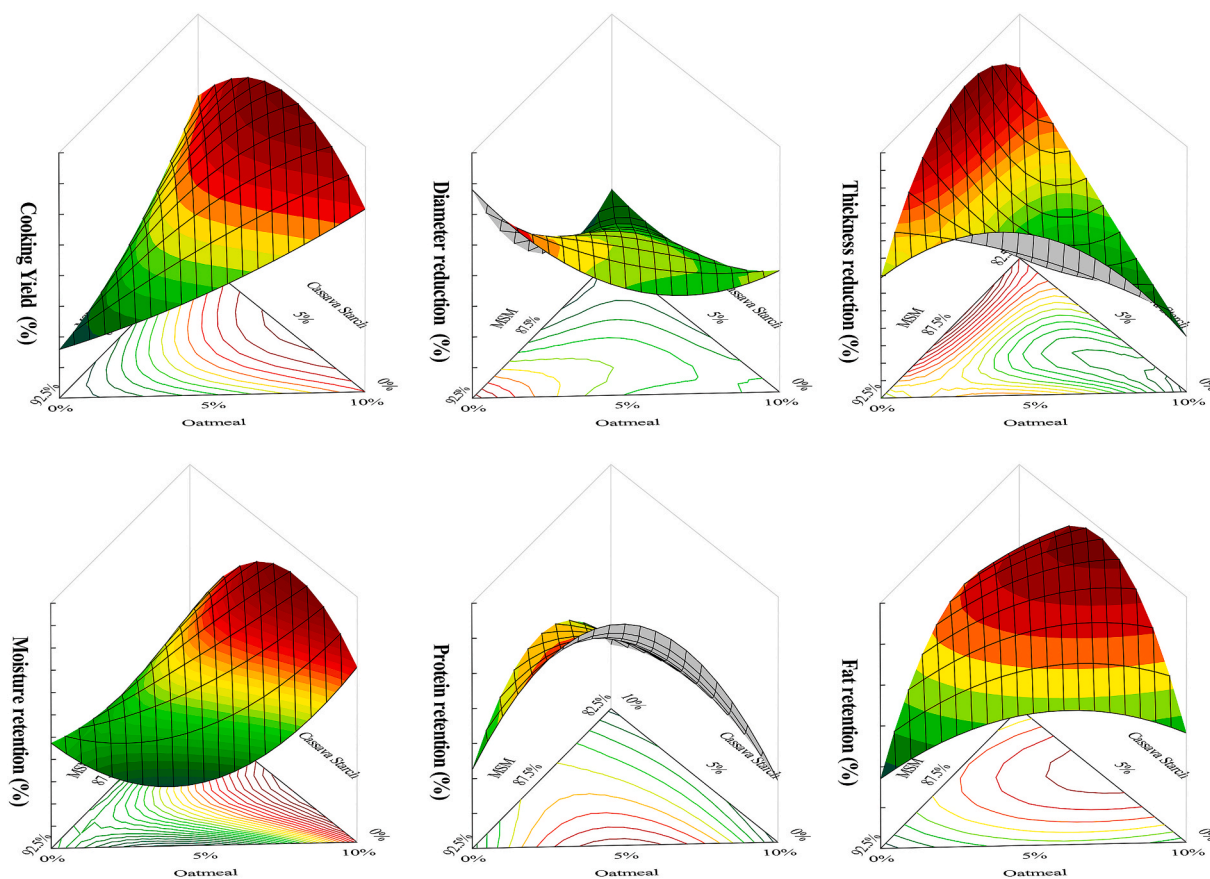


Fig. 3. Response surface plots for effects of oatmeal and cassava starch on cooking properties of fish burger formulations.

and b^* values were found for the blended fish burger with different percentages of shrimp, camel, and ostrich (Shekarabi et al., 2020).

Other studies that used white meat fish also presented correlated values, such as the study by Raúl et al. (2018) with the addition of different wheat bran in fish burgers of biquara (*Haemulon plumieri*), and fish burgers produced with different levels of filleting catfish (*Rhamdia quelen*) (Bochi et al., 2008). According to Cilli et al. (2019) in the evaluation of effects of grape pomace flour on quality parameters of salmon burgers (*Salmo salar*), observed higher values for L^* and a^* and lower values for b^* . The addition of different ingredients tends to modify the food color. The different flour concentrations indicated a decrease in the L^* band and an increase in the a^* and b^* bands. However, the changes were not significant between formulations ($p \geq 0.05$). This result may have been influenced by the composition of the main ingredients, such as MSM, oatmeal, and cassava starch, which remain in the same color spectrum.

3.4. Technological cooking properties

Samples of the raw and grilled fish burgers were measured (Fig. 2). Significant effects of the variables on the cooking properties of the fish burger were evidenced from the response surface (Fig. 3). The addition of oatmeal and cassava starch favors the yield and retention of nutrients, especially in the combination between them. Verification of the cooking process is essential for foods of animal origin since micro and macro-nutrients change when subjected to heat treatment. Some of the water evaporates, bonds tend to break, and other compounds denature, resulting in nutritional transformation and economic loss (Sheard, Nute, & Chappell, 1998).

The cooking yield results showed a synergistic effect with better performance in samples with the addition of the two flours (F6:O5C5) ($p \leq 0.05$). A similar result regarding the inclusion of flours in fish burgers was observed in previous studies, such as the addition of chitosan in Nile tilapia fish burgers (*Oreochromis niloticus*) (Farias, Ambrósio, Vieira, Menezes, & César, 2019), and in the study with the addition of oatmeal and cassava starch in the fish burger with Nile tilapia pulp (*Oreochromis niloticus*) (Braga et al., 2008). However, these results are not observed in all types of flour. In a study with the addition of wheat bran to biquara fish burgers (*Haemulon plumieri*), observed an effect of loss on yield and reduction when the addition was greater than 1% of wheat bran (Raúl et al., 2018).

The metric aspects of diameter and thickness reduction were also influenced by the addition of 10% flour (F2:O10; F3:C10). The response surface indicated inverse curves, while oatmeal affected the diameter reduction, cassava starch influenced the thickness reduction. Similar results were observed in studies with the addition of yacon flour to fish burgers of Nile tilapia (*Oreochromis niloticus*) (Zitkoski, Vendruscolo, Kuanei, Pinto, & Bairy, 2019).

There was no significant difference in moisture retention between the formulations ($p \geq 0.05$). Fat retention improved with the addition of both flours, and it performed better when combining both (F6:O5C5) ($p \leq 0.05$). Protein retention differed only in the formulation F4:O5C0 ($p \leq 0.05$) with better performance. However, when more oatmeal was added, there was a reduction in performance (F2:O10C0). The results on retention were better than those evaluated in the formulation of fish burgers with filleting waste pulp catfish (*Rhamdia quelen*) (Bochi et al., 2008). The moisture retention was similar to the study on the texture of fish burgers of Nile tilapia (*Oreochromis niloticus*), whereas fat retention values were lower (Bainy et al., 2015).

3.5. Optimization of multiple parameters

In this study, it was possible to predict significant effects on several factors, such as proximate composition, textural, and cooking properties. The special cubic response model allowed us to assess the influence of variables on the responses. In addition, the analysis of variance

Table 5

Analysis of variance of the experimental responses for MSM-based fish burgers obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Dependent variable response	SS	MS	R ²	p-Value
Moisture (%)	137.2482	22.8747	0.9823	0.0000*
Ash (%)	0.3349	0.0558	0.8472	0.0000*
Fat (%)	4.2788	0.7131	0.8520	0.0000*
Protein (%)	6.2939	1.0490	0.6735	0.0026*
Carbohydrate (%)	149.4580	24.9097	0.9672	0.0000*
pH	0.0095	0.0016	0.4131	0.1204 ^{ns}
aw	0.0000	0.0000	0.3291	0.2567 ^{ns}
Cooking yield (%)	332.0694	55.3449	0.8639	0.0000*
Diameter reduction (%)	182.8260	30.4710	0.7825	0.0002*
Thickness reduction (%)	761.0008	126.8335	0.5934	0.0117*
Moisture retention (%)	108.2728	18.0455	0.4209	0.1112 ^{ns}
Fat retention (%)	145.5693	24.2615	0.7399	0.0005*
Protein retention (%)	208.4370	34.7395	0.7946	0.0001*
Hardness	738.8190	123.1365	0.6413	0.0050*
Cohesiveness	0.0289	0.0048	0.5552	0.0212*
Springiness	0.0487	0.0081	0.8630	0.0000*
Chewiness	245.6619	40.9437	0.6799	0.0023*
L^*	102.3933	17.0655	0.2301	0.5082 ^{ns}
a^*	16.1247	2.6875	0.1036	0.8764 ^{ns}
b^*	106.0128	17.6688	0.1380	0.7856 ^{ns}

SS sum of squares, MS mean square. Statistical significance: * $p \leq 0.05$, ^{ns} $p > 0.05$.

(ANOVA) confirmed the significance of the model's impact on these responses (Table 5).

As the objective of this study was to verify the inclusion of oatmeal and cassava starch in the fish burger formulation, the texture parameters and cooking technological properties were criteria for optimization. As the response surface for the F6:O5C5 formulation presented results close to the optimal points, the predicted values were validated with this formulation (Table 6). The optimization results will indicate the influence of the mixture on the beneficial effect on the technological properties of cooking. The lower limit and upper limit for the response parameters of the dependent variables were set at levels that ensured the integrity and intrinsic characteristics of the product.

Previous studies address the strong influence and improvements in the textural and yield of the fish burger when flour and starches are added (Braga et al., 2008; Coelho et al., 2007). This same effect was verified in studies with conventional products of animal origin, attenuating losses and shortening during cooking and adding juiciness (Guedes-Oliveira, Salgado, Costa-Lima, Guedes-Oliveira, & Conte-Junior, 2016; Ryszard Rezler, Krzywdzińska-Bartkowiak, & Piątek, 2021). According to Talukder (2015), there are nutritional advantages to the addition of these ingredients, especially when they are used as fat substitutes, in addition to presenting an economic gain, as they are products of lesser value compared to products of animal origin. The F6:O5C5 formulation showed a 9.69% reduction in the cost of ingredients compared to the formulation without the addition of flour, at a cost of around 7.85 dollars (USD) per kg.

3.6. Quantitative descriptive analysis (QDA)

Sensory evaluation is a tool that allows interpreting responses to organoleptic senses and is one of the main evaluation mechanisms of food products (Dutcosky, 2011). For sensory validation, all studied formulations were analyzed under the 12 attributes raised, and the results of the quantitative descriptive analysis of the formulations are indicated in Fig. 4.

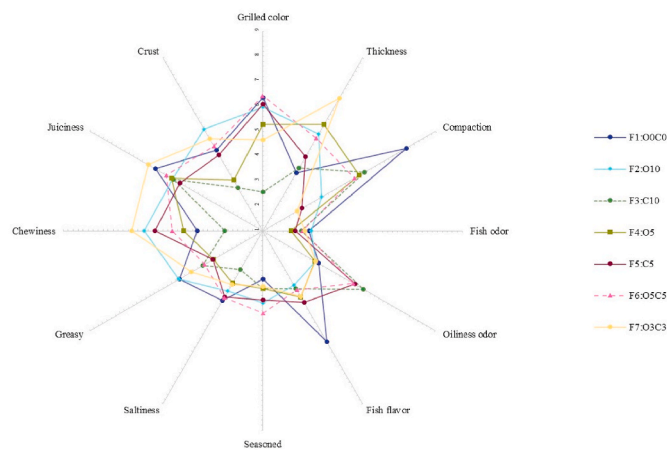
Interactions between independent variables significantly influenced the palatability of the product ($p \leq 0.05$). This result was expected, since the addition of different ingredients tends to modify the intrinsic characteristics, with effects on sensory characterization. Other studies that analyzed different concentrations of ingredients in fish burgers also

Table 6

Predicted and observed values for optimization parameters of the fish burger formulation.

Dependent variable response	Goal	Lower limit	Upper limit	Predicted value	Experimental value (F6:O5C5)	Relative Error (%)
Cooking yield (%)	Maximize	70	100	85.41	89.38 ± 0.75	2.32
Diameter reduction (%)	Minimize	0	20	5.54	2.62 ± 0.88	41.22
Thickness reduction (%)	Minimize	0	30	18.08	23.71 ± 2.90	28.05
Moisture retention (%)	Maximize	70	100	91.13	93.53 ± 1.68	2.17
Fat retention (%)	Maximize	80	100	93.62	99.59 ± 0.29	1.18
Protein retention (%)	Maximize	80	110	99.05	99.28 ± 1.68	3.20
Hardness (N)	Is in range	10	60	34.05	24.21 ± 2.23	20.16
Chewiness (N·cm)	Minimize	5	30	13.83	9.39 ± 0.93	1.60

Results are means ± standard deviation.

**Fig. 4.** Descriptive Qualitative Analysis in a radar chart, for the attributes of appearance, aroma, flavor, and texture in fish burger formulations added of oatmeal and cassava starch.**Table 7**Sensory profile of the affective analysis of MSM-based fish burgers obtained from tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch.

Form.	Global acceptance (9-Point hedonic Scale)	Purchase intention	Preference ranking*
F1: O0C0	5.67 ± 1.67 ^a	2.75 ± 0.96 ^a	5.42 ± 1.35 ^b
F2:O10	6.92 ± 1.26 ^a	3.25 ± 1.13 ^a	3.58 ± 1.92 ^{ab}
F3:C10	6.50 ± 1.25 ^a	2.83 ± 0.89 ^a	4.75 ± 1.75 ^b
F4:O5	6.17 ± 1.03 ^a	3.08 ± 0.63 ^a	4.25 ± 1.13 ^{ab}
F5:C5	6.25 ± 1.17 ^a	3.33 ± 0.83 ^a	3.92 ± 1.75 ^{ab}
F6: O5C5	7.58 ± 0.92 ^a	3.83 ± 0.72 ^a	3.00 ± 1.33 ^a
F7: O3C3	6.75 ± 1.50 ^a	3.67 ± 0.78 ^a	3.08 ± 1.61 ^a

Results are means ± standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).*The critical difference was evaluated with the Friedman method ($p \leq 0.05$).

showed similar results on appearance, odor, flavor, and texture (Pictures, Rocha, Ferreira, & Bolini, 2015; Ali, Mansour, E-Ibedawey, & Osheba, 2019).

The addition of flour had a significant effect on thickness, fish flavor, and compaction compared to the formulation without flour (F1:O0C0) ($p \leq 0.05$). Oatmeal had a greater effect on crust addition and thickness perception, while cassava starch had a reduction in grilled color, chewiness, and crust. However, the binary and ternary mixtures (F6: O5C5, F7: O3C3) indicated a synergistic effect on juiciness and thickness. The influence of the addition of flour was greater on appearance and texture. The study by Raúl et al. (2018), also found a greater influence on the texture and color of fish burgers of biquara (*Haemulon plumierii*) with the addition of different wheat white concentrations.

plumierii) with the addition of different wheat white concentrations.

The results of descriptive parameters on fish odor, saltiness, and greasy did not show any significant difference between samples ($p \geq 0.05$).

3.7. Sensory affective testing

The results of the affective evaluation are presented in Table 7. The means of global acceptance did not show any significant difference ($p \geq 0.05$). The formulation F6:O5C5 presented the best score, being in the range between “liked moderately” and “liked a lot”, the other formulations showed homogeneity, in the range of “liked slightly”. Similar results were found in previous studies with convenience products based on tambaqui (*Colossoma macropomum*), Lima et al. (2020) obtained a score of 7.35 in the sensory evaluation of tambaqui fish meatballs, and according to Anjos et al. (2021), the tambaqui fish burger acceptability index was higher in the formulations enriched with green banana and chitosan biomass. The addition of oatmeal and cassava starch showed an improvement in the global acceptance of 7.44 and 7.26, respectively, in the fish burger of Nile tilapia pulp (*Oreochromis niloticus*) (Raga, Pasquetti, Bueno & Merengoni, 2008). According to Dutcosky (2011), the global acceptance test using a 9-point hedonic scale is one of the main items of sensory analysis, seeking to assess the relationship between the product/consumer and its acceptance.

In the purchase intention test, the values were between “I would buy whenever I had the opportunity” and “I would buy if I had access”, the results showed no significant difference ($p \geq 0.05$). Values higher than 3.87 to 4.36 were found with the tambaqui fish burger (*Colossoma macropomum*), enriched with green banana biomass and chitosan (Anjos et al., 2021), and similar results were found in the study by Raúl et al. (2018), with fish burger of biquara (*Haemulon plumierii*) with the addition of different wheat white concentrations, where the purchase intention was between 2.41 and 3.50.

The means of the preference ordering test showed a significant difference ($p \leq 0.05$). Formulation F6:O5C5 obtained the best position in the ranking, while the formulation without the addition of flour obtained the worst position (F1:O0C0).

The results of the sensory evaluation showed that fish burgers based on MSM of tambaqui (*Colossoma macropomum*) with the addition of oatmeal and cassava starch were well accepted, with positive performance on the attributes of appearance, odor, flavor, texture, and hedonic tests.

4. Conclusion

The use of the simplex-centroid mixture design was satisfactory to estimate the effect of the pseudo-components and estimate the optimal formulation. According to the results above, the addition of oatmeal and cassava starch in the fish burger formulation showed beneficial effects on the textural, functional, and sensory properties, with desirable attributes in terms of appearance and advantages in nutritional aspects, such as the addition of fiber in the product of animal origin. Regarding optimization, the formulation with a binary combination of two flours

showed results with better adjustment to the predicted optimal values, having a positive influence on the acceptance, cooking technology properties, in addition to cost reduction, as they are low-value ingredients.

The use of tambaqui MSM was satisfactory for the development of conventional products as a strategy to promote the full use of fish, and the addition of alternative ingredients can contribute to the improvement of functional and sensory properties.

CRediT authorship contribution statement

Leandro Presenza: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Luis Felipe de Freitas Fabrício:** Methodology, Execution of experiments, Writing – original draft. **Juliana Antunes Galvão:** Methodology, Writing – review & editing. **Thais Maria Ferreira de Souza Vieira:** Conceptualization, Supervision, Responsible for financial support, Formal analysis, Writing – review & editing.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2021.113008>.

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