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RESEARCH PAPER

Cryoprotective Agents to Improve the Quality of Tambaqui Surimi (*Colossoma macropomum*)

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Abstract

One major method in fish processing to maximize utilization is through surimi preparation, extensively used in various products. To ensure desired organoleptic qualities, cryoprotective agents are crucial. This study aimed to evaluate the production potential of Amazon fish surimi prepared with different blends of cryoprotectants: CF (no cryoprotectant), S1 (2% sodium chloride, 1% sucrose), S2 (8% sorbitol, 0.5% sodium tripolyphosphate), and S3 (8% sucrose, 8% sorbitol, 0.5% sodium tripolyphosphate). Proximal composition and techno-functional properties were assessed. Moisture content (<79%) indicated excellent quality across all treatments. S2 had high protein (17.91%) and low lipid content, second only to S3. Increasing cryoprotectant concentration led to higher carbohydrate content and energy value. Color differences were insignificant except for luminosity (L*) influenced by sucrose in S1. Cryoprotectant treatments showed no significant texture or water retention differences, displaying a bending profile well-suited for the product. Univariate statistics and Principal Component Analysis concluded S2 blend as most suitable for tambaqui surimi (*Colossoma macropomum*) production regarding final nutritional and techno-functional gel characteristics.

Introduction

Surimi, an ancient practice for improving the utilization and preservation of fish, has considerable industrial importance in the fisheries sector and has been produced for many years (Cabral *et al.*, 2008; Zhao & Shen, 2016). The industrial production of surimi begins by washing ground or minced fish meat in cold potable water to solubilize compounds, such as sarcoplasmic proteins and enzymes, as well as remove possible impurities, such as skin residues and fat (Brasil, 2020; Park, 2005). The washing stage enhances the brightness and whiteness to the product, in addition to remove volatile compounds that give a fishy odor, thus assisting in stability during cold storage (Neiva & Gonçalves, 2011). The washing cycles are repeated several times and excess water is removed by pressing.

Next, a meat paste that is rich in myofibrillar proteins is obtained, and low-molecular-weight ingredients, called cryoprotectant agents, are added. These agents prevent protein degradation and the rupture of muscle cells due to the action of ice crystals formed during freezing, which is the main mode of surimi storage (Walayat *et al.*, 2022). Additionally, cryoprotectant agents function in protein gelation, making surimi a basic ingredient in the production of different foods such as kani-kama, kamaboko, ham, fish burgers, and sausages (Fogaça *et al.*, 2015; Park, 2005).

Numerous cryoprotectants are used in surimi production, with the most commonly used ones being those with a lower cost of acquisition and greater versatility concerning final gel quality. Among these, sodium chloride, sucrose, sorbitol, and sodium tripolyphosphate have been reported. These agents confer techno-functional properties to surimi, such as maintaining the protein structure and forming a stable gel during freezing, as well as increasing the water retention capacity and contributing to an improvement in the sensory quality of the final product (Bruno *et al.*, 2020; Fogaça *et al.*, 2015; Rezende-de-Souza *et al.*, 2020; Walayat *et al.*, 2022).

In Brazil, tambaqui (*Colossoma macropomum* Cuvier 1818), the highest-produced fish (Peixes BR, 2024), is native to the Amazon region and is cultured in freshwater, has been the subject of research for the production of convenient fish-based products. In the literature there are reports that this species has approximately 16% protein and 2.25 to 18.59% fat (Mesquita *et al.*, 2018; Roa *et al.*, 2019), with this variation depending on the type of food provided to the animal, growth phase and type of production system. Its musculature of this species is composed of ordinary and blood muscles, with moisture and protein contents close to each other, 78.6% moisture and 19.3% protein for ordinary muscles, and 76.2% moisture and 17.9% protein for blood muscles (Souza *et al.*, 2018). However, there is limited information regarding the use of this species in surimi production and the evaluation of its nutritional and technological qualities (Bruno *et al.*, 2020; Rezende-de-Souza *et al.*, 2020). This study aimed to evaluate the nutritional potential and techno-functional properties of tambaqui surimi using different blends of cryoprotective agents.

Materials and Methods

Preparation and Characterization of the Raw Material

Tambaqui (*C. macropomum*) meat was purchased from a local shop for surimi production, in Mato Grosso, Brazil. Before preparing the surimi samples, samples were collected to assess the freshness and characterize the raw material. The analyses included pH measurement (Zenebon *et al.*, 2008) and quantification of total volatile basic nitrogen (TVB-N) (Savay-da-Silva *et al.*, 2008), and the results were compared with the freshness limits determined in Brazilian Decree Nº. 10.468/2020 (Brasil, 2020).

A proximate composition analysis was also performed, including total moisture content determined by drying in an oven at 105°C until a constant weight was achieved; crude protein content determined by the micro-Kjeldahl method using a correction factor of 6.25; total lipid content determined by the Soxhlet extraction method using petroleum ether as the solvent; and total ash content determined by complete incineration of the sample in a muffle furnace at 550°C (Brasil, 2011). The carbohydrate composition and total energy value (TEV) were obtained using Equations 1 and 2 (Brasil, 2003). The results were expressed on a wet basis.

$$\text{Carbohydrate (\%)} = 100 - \text{protein} - \text{lipid} - \text{ash} \quad \textit{Equation 1}$$

$$\text{Energy value (kcal/100g} = ((\text{protein}+\text{carbonhyrate})*4)+(\text{lipid}*9) \quad \textit{Equation 2}$$

Elaboration of the Surimi

The triturated tambaqui meat was washed five times with potable water at 5±2°C, following the procedure described by Rezende-de-Souza *et al.* (2020). In the first washing cycle, sodium bicarbonate (Daxia Ingredientes and Aditivos, São Paulo, Brazil) was added until the fish-water mixture was neutralized (pH 7.0). From the second to the fourth cycle, only water was used for washing, whereas in the fifth washing cycle, 1% sodium chloride was added to the mixture in relation to the weight of the fish meat; the salt was used in the last washing cycle to intensify the solubilization of myofibrillar proteins, especially myosin, and contribute to the formation of the surimi gel (Walayat *et al.*, 2022). Excess water was removed between washing cycles using a voile fabric and a semi-manual press. After the last washing cycle, a light-colored paste was obtained (L^*=78.38), which was divided into four parts, one of which was for the control treatment (without the addition of cryoprotectants), and the other three were distributed among the treatments with different formulations of cryoprotectants. Cryoprotectant blends were selected based on studies conducted using surimi from other species (Table 1).

Once the cryoprotective agents were added to the fish paste, homogenization was performed using a food processor (HC31, Black+Decker®, Maryland, USA) for 150 s; during this process, all the ingredients were at 0ºC. Subsequently, the mixture was vacuum packed and frozen in an IULT335D ultra-freezer (Indrel Scientific, Paraná, Brazil) at -80°C for 30 days. The freezing stage was applied so that the surimi gels could be formed and, after this period, the surimis were thawed in an LT320T incubator (LimaTec, Bahia, Brazil) at 2±1°C for 24 h, and then subjected to laboratory analysis.

Characterization of Surimi

In the surimis, the proximal composition (moisture, crude protein, total lipid, total ash, and carbohydrate) and total energy value (TEV) were determined as described previously. Colorimetric evaluations of lightness (L^*), redness (a^*), and yellowness (b^*) were conducted using a CR-400/410 colorimeter (Konica Minolta, Osaka, Japan) with a standard observer of 10°, D65 illuminant, and CIELab color system. Chromaticity (C*) and hue (h*) values were calculated (Rezende-de-Souza *et al.*, 2020). Additionally, gel quality assessments

Table 1. Formulation of cryoprotective blends in tambaqui surimi (*C. macropomum*)

Ingredient (%)	CF	S1	S2	S3
Sodium chloride (commercial)	NA	2.0	NA	NA
Sucrose (commercial)	NA	1.0	NA	8.0
Sorbitol (DAXIA)	NA	NA	8.0	8.0
Sodium tripolyphosphate (DAXIA)	NA	NA	0.5	0.5
Species used	*Colossoma macropomum*	*Oreochromis niloticus*	*Colossoma macropomum*	*Brycon cephalus*
Reference	NA	Fogaça *et al.* (2015)	Rezende-de-Souza *et al.* (2020)	Vasconcelos *et al.* (2016)

NA, not applicable; CF, control formulation (without cryoprotection); S1, 2% sodium chloride and 1% sucrose; S2, 8% sorbitol and 0.5% sodium tripolyphosphate; S3, 8% sorbitol, 8% sucrose and 0.5% sodium tripolyphosphate

were performed, including analysis of water-holding capacity (WHC) (Hamm, 1961); shear force (SF) with samples of 2.5 cm length × 1.0 cm width × 1.0 cm height, using a TA.XT PlusC texture analyzer (Stable Micro Systems, São Paulo, Brazil) configured at 1.0 mm/s for the test, 2.0 mm/s for the post-test, and 10.0 mm/s for the pre-test, with the assistance of a shear probe (Fogaça *et al.*, 2015, modified). Also evaluated the foldability capacity test, following the methodology described by Olivares and Castro (2001), using slices of surimi 35 mm in diameter and 3 mm thick, which are classified as AA when they do not break when folded into four parts; A when they break slightly when folded into four parts, but do not split when folded in half; B if they break slightly when folded into two parts; and C when they break when left in halves, but do not split.

Data Analysis

Analyses were performed with seven replicates per treatment, and the results were analyzed using R software, version 4.3.1 (New Zealand). Descriptive statistics were used to characterize the raw materials. The effect of treatment on the surimi response variables (moisture, protein, lipid, ash, carbohydrate, TEV, *L*, a*, b**, *C**, *h**, SF, and WHC) was determined using Gamlss models with a normal distribution, an identity link function for the mean, and a logarithmic link function for the standard deviation (Rigby & Stasinopoulos, 2005). The significance of the parameter estimates and differences between means were evaluated using the Wald test at a 5% probability level with Bonferroni correction for multiple comparisons. The residual analysis for each model was performed using a "worm plot" graph, and a result was considered adequate when at least 95% of the residuals were within two elliptical curves and close to the x-axis.

Using Matlab 2019 software (The MathWorks Inc., Natick, USA), Pearson's correlation test was performed to check for linear correlation between the physicochemical parameters with a *P*-value to determine the statistical significance of this correlation; correlations were interpreted as negligible ($r<0.29$), weak (r values between 0.3 and 0.49), moderate (r values between 0.5 and 0.69), strong (r values between 0.7 and 0.9), and very strong ($r>0.91$). In addition, the Principal Component Analysis (PCA) was performed to detect patterns in the treatments with different cryoprotectant blends.

Results

Raw Material Characterization

The raw material exhibited an average of pH 6.20 and TVB-N value in muscle tissue found 11.53 mg/100g. The proximal composition of the raw material shows satisfactory conditions for the species (Table 2). However, it is important to highlight the lipid content of 4.75 g/100g, as it is the main constituent to be eliminated in the washing processes for surimi production.

Surimi Nutritional and Techno-functional Properties

Humidity decreases significantly with the addition of cryoprotectants. S2 and S3 had the lowest humidity values, followed by S1 and finally CF (Table 3). However, all treatments are considered to be of good quality according to their moisture content.

Owing to dilution, the total protein and total lipid contents decreased significantly after the addition of cryoprotectants compared to the control formulation (Table 3). The highest protein content was for CF, which differed significantly only from S1 and S3, which had the lowest values for total protein. Among the treatments with cryoprotectants, S3 had the lowest lipid value compared to S2, while S1 did not differ from S2 and S3. However, CF had the highest lipid content of all the treatments evaluated.

The total ashes content was significantly different between all the treatments (Table 3). S1 was the treatment with the highest ashes concentration, reaching almost 3%; while S2 had just over 1%, a higher concentration than the 0.82% for S3, all of which were higher than the control formulation (0.62%).

The blends of cryoprotective agents significantly affected the total carbohydrate content in all the treatments evaluated, this being the chemical group most affected in the entire proximal composition (Table 3). CF was the one with the lowest carbohydrate concentration, with 2.14%, followed by S1 (6.16%), S2 (14.46%) and finally S3 (17.92%). In terms of CF composition, S1, S2 and S3 had a total increase of 187.53%, 575.70% and 737.38%, respectively (data not shown in table).

Due to the various changes in proximal composition, the total energy value was also affected ($P<0.05$). S2 and S3 were those with the highest TEV,

Table 2 Mean (± standard deviation) of proximal composition (g.100g^{-1}) and energy value (kcal.100g^{-1}), on a wet basis, of tambaqui fillet (*C. macropomum*) compared to the literature.

Parameter	Data from this study	Ramos *et al.* (2016)	Liebl *et al.* (2021)	Cavali *et al.* (2021)
Moisture	76.73±0.43	79.0	76.16	75.54
Proteins	17.36±0.35	18.07	18.26	17.64
Lipids	4.75±0.14	1.10	2.53	5.74
Ashes	1.05±0.02	0.91	1.28	1.06
Carbohydrates	0.10±6.68	0.92	*	*
Energetic value	112.71±1.73	85.87	*	*

* Data not presented in original paper.

Table 3. Proximal composition (g.100g^{-1}) and energy value (kcal.100g^{-1}), on a wet basis, of tambaqui surimi (*C. macropomum*) prepared with different blends of cryoprotectants.

Parameter	CF	S1	S2	S3
Moisture	75.74±0.28 ^{a}	72.42±0.45 ^{b}	65.54±0.42 ^{c}	64.89±0.63 ^{c}
Protein	18.82±0.08 ^{a}	17.42±1.07 ^{bc}	17.91±0.83 ^{ab}	16.14±0.89 ^{c}
Lipid	2.67±0.47 ^{a}	0.96±0.32 ^{bc}	1.14±0.11 ^{b}	0.63±0.35 ^{c}
Ashes	0.62±0.06 ^{d}	2.97±0.13 ^{a}	1.11±0.06 ^{b}	0.82±0.05 ^{c}
Carbohydrate	2.14±0.53 ^{d}	6.16±1.12 ^{c}	14.46±0.88 ^{b}	17.92±0.96 ^{a}
Energy value	107.92±2.68 ^{b}	102.96±2.26 ^{c}	138.90±2.30 ^{a}	140.21±3.45 ^{a}

^{a,b,c} Means (± standard deviation) estimated by Gamlss models, with different letters on the same line, differ by the Wald test, with Bonferroni correction, at the 5% significance level (N = 7). CF, control formulation without cryoprotection; S1, 2% sodium chloride and 1% sucrose; S2, 8% sorbitol and 0.5% sodium tripolyphosphate; S3, 8% sorbitol, 8% sucrose, and 0.5% sodium tripolyphosphate.

with no difference between them. S1, on the other hand, had the lowest TEV, below even CF.

The hue, saturation and redness and yellowness were not significantly affected by the different blends of cryoprotective agents (Table 4). The hue was close to 90, indicating a yellow color to the products. This is confirmed by the higher *b** values (5.85-6.89) than *a** values (0.67-1.03), resulting in a predominance of yellow over red. However, there was low saturation, indicating a lack of real color in the products. Therefore, the surimis were colorless due to their tendency to turn white. What confirms this are the luminosity values, which were above 65, values that tend towards white. Brightness was the only colorimetric parameter that was influenced by the treatments ($P<0.05$) (Table 4). S1 was the treatment with the highest degree of whiteness, while S3 was the darkest as it had the lowest *L** value. S2 and CF did not differ from each other, but were different from the other treatments.

The force required to shear the samples did not differ ($P>0.05$). However, water holding was significantly influenced by the different blends of cryoprotective agents (Table 5). S1 and S2 had the highest water holding rates, while CF had the lowest. S3, on the other hand, did not differ from any other treatment.

The foldability capacity test classifies samples according to their folding capacity. Therefore, treatments CF, S1, S2, and S3 were classified as C, A, A, and AA, respectively. Classes AA are better than A, which in turn are better than class C. Thus, the surimis with different cryoprotective agents blends presented better folding conditions compared to CF.

Correlation between Quality Parameters

Correlations were observed between nutritional attributes and those related to the color of the different tambaqui surimi, with both strong and moderate correlations, negative or positive, but all significant. The correlations relating to energy value, for example, were more closely related to moisture content (r=-0.929) and carbohydrate content (r=0.904). However, shear force and water retention capacity are among the most important quality indicators of the surimi gels presented in this study. Nevertheless, shear force showed no correlation with the other quality parameters evaluated in the different tambaqui surimis, with r values below 0.3. With regard to water retention, positive and weak correlations were observed when compared with moisture content (r=-0.435), lipid content (r=-0.470) and mineral content (r=0.447) (Figure 1).

The variability of the presented data was explored through PCA (Figure 2). The first two PCs explained 58.55% of the data variability concerning the analyzed parameters in the different surimi treatments. PC1, which accounted for 36.46% of the explained variance, was primarily responsible for the clustering of samples in the treatments. Samples CF and S1 were located in the positive region of PC1, indicating their similarities. However, variables such as moisture, protein, lipids, and luminosity (*L**) contributed more significantly to CF, while coordinates for yellow (*b**) and red (*a**) colors, as well as saturation (*C**) and total ash, were more strongly associated with S1. In the negative region of PC1, the highest absolute loading values corresponded to the total carbohydrate content and energy values, which

Table 4. Colors of tambaqui surimi (*C. macropomum*) prepared with different blends of cryoprotectants

Parameter	CF	S1	S2	S3
h*	82.65±3.47 [a]	81.39±6.62 [a]	84.75±4.15 [a]	83.70±3.31 [a]
a*	0.90±0.50 [a]	1.03±0.86 [a]	0.67±0.58 [a]	0.68±0.36 [a]
b*	6.48±0.49 [a]	5.85±0.57 [a]	6.89±1.03 [a]	6.06±0.73 [a]
C*	6.7±0.74 [a]	5.94±0.52 [a]	6.94±1.06 [a]	6.11±0.74 [a]
L*	78.38±0.77 [b]	80.77±1.55 [a]	77.11±1.58 [b]	68.18±1.62 [c]

[a,b] Means (± standard deviation) estimated by Gamlss models, with different letters on the same line, differ by the Wald test with Bonferroni correction at the 5% significance level (N=7). CF, control formulation without cryoprotection; S1, 2% sodium chloride and 1% sucrose; S2, 8% sorbitol and 0.5% sodium tripolyphosphate; S3, 8% sorbitol, 8% sucrose, and 0.5% sodium tripolyphosphate; h*, hue; a*, red-green coordinate; b*, yellow-blue coordinate; C*, saturation; L*, luminosity.

Table 5. Shear Force (N) and water-holding capacity (%) of tambaqui surimi (*C. macropomum*) prepared with different blends of cryoprotectants

Parameter	CF	S1	S2	S3
Shear Force	11.36±5.11 [a]	14.07±3.82 [a]	10.98±3.44 [a]	12.42±3.66 [a]
Water Holding	76.95±0.46 [b]	85.22±3.32 [a]	88.15±1.35 [a]	81.71±4.71 [ab]

[a,b] Means (± standard deviation) estimated by Gamlss models, with different letters on the same line, differ by the Wald test with Bonferroni correction at the 5% significance level (N=7). CF, control formulation without cryoprotection; S1, 2% sodium chloride and 1% sucrose; S2, 8% sorbitol and 0.5% sodium tripolyphosphate; S3, 8% sorbitol, 8% sucrose, and 0.5% sodium tripolyphosphate.

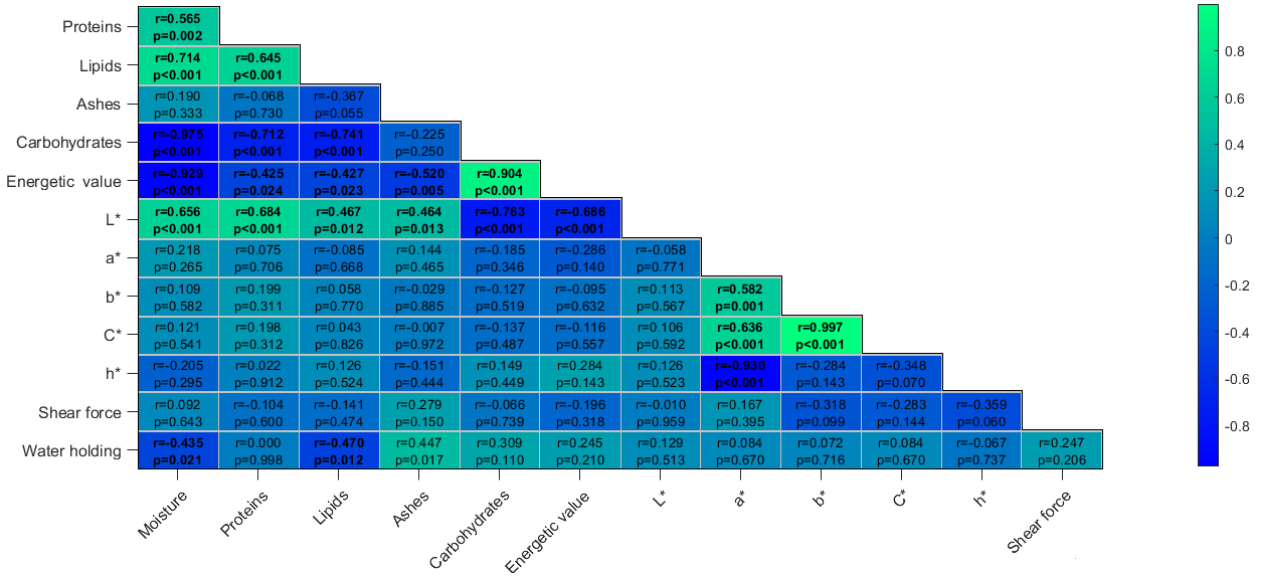

Figure 1. Pearson's correlation of the nutritional and techno-functional properties of tambaqui surimi (*C. macropomum*) prepared with different blends of cryoprotectants.

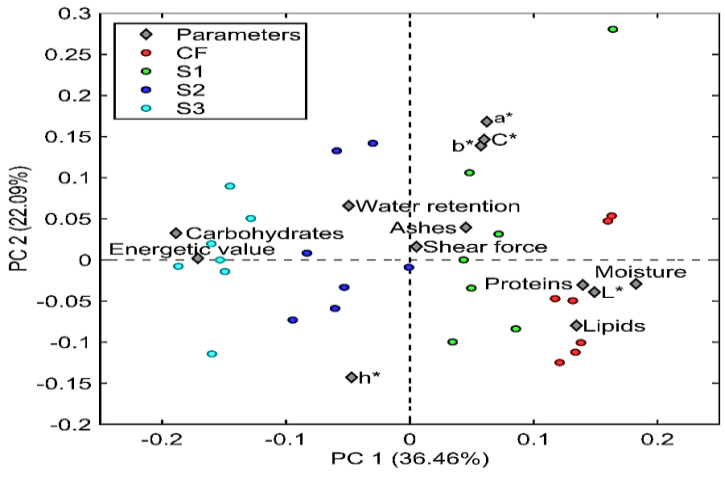

Figure 2. Principal Component Analysis of the nutritional and techno-functional properties of tambaqui surimi (*C. macropomum*) prepared with different blends of cryoprotectants.
CF, control formulation (without cryoprotection); S1, 2% sodium chloride and 1% sucrose; S2, 8% sorbitol and 0.5% sodium tripolyphosphate; S3, 8% sorbitol, 8% sucrose and 0.5% sodium tripolyphosphate.

were mainly associated with S3. S2 showed some resemblance to the samples in the S3 treatment, but it was more closely associated with variables related to water-holding capacity and *h** values. The shear force variable, however, had a loading close to 0, indicating a low influence on sample clustering.

Discussion

Raw Material Characterization

The pH and TVB-N values categorize the raw material as a fresh product suitable for use in surimi production, as stipulated by current Brazilian legislation (Brasil, 2020). This legislation sets maximum values for freshness indicators, with pH=7.0 and TVB-N<30 mg/100g. The values obtained for the proximal composition parameters and total energy values were satisfactory and consistent with the species characteristics and are presented as a reference for possible interpretations of the composition of the surimis (Table 2). Within the same species, variations in fish meat composition can be attributed to various factors, including animal size and diet (Cavali *et al.*, 2021; Liebl *et al.*, 2021).

Surimi Nutritional and Techno-functional Properties

The reduced the moisture content was attributed to the addition of solids to the fish paste, resulting in a dilution of the total moisture content. Formulations S2 and S3 had no differences, which could be attributed to the use of sodium tripolyphosphate. Phosphate has buffering properties that contribute to the depolymerization of thick filaments and the partial expulsion of water from the matrix (Walayat *et al.*, 2022; Xiong *et al.*, 2000). In surimi, one of the main quality indicators during processing is moisture content, as excessively high levels can result in a sticky mass that is difficult to process for the production of its derivatives. Therefore, a classification was proposed for Alaska pollock (*Theragra chalcogramm*a) surimi, where higher moisture levels classify surimis as Class A (79.1–80.0%), Class B (80.1–81.5%), and Class C (>81.5%) (Suzuki, 1981). Thus, the different surimi formulations in this study fall within this superclass, as they exhibit a moisture content of up to 79.0%.

The protein composition of the tambaqui surimi in this study was higher than that reported in the literature. In tilapia (*Oreochromis mossambicus*) surimi, the protein content was reported to be 14.83% (Seighalani *et al.*, 2017) and 16.49% (Majumder *et al.*, 2017), while for silver carp (*Hypophthalmichthys molitrix*) it was 12.53% (Liang *et al.*, 2020). In tambaqui (*C. macropomum*) surimi that had been through five washing cycles, the protein content varied from 13.84% to 15.51% (Fogaça *et al.*, 2018), depending on the concentration of starch added to the formulation. These variations between species and between studies are related to the processing used to obtain the surimi; however, the lower protein values reported in the literature correspond to the final composition of the surimi. This is because in different studies other ingredients are used in the composition, such as starches and gums. For this study, only the cryoprotective agents were added to the surimi.

Lipids are of low technological importance for surimi. There was a decrease in lipid content after the addition of cryoprotective agents, due to the dilution of this macromolecule in the surimi matrix. Its concentration tends to be close to 1%, and this behavior has also been observed in different studies with surimi obtained from different species (Liang *et al.*, 2020; Majumder *et al.*, 2017; Seighalani *et al.*, 2017).

With the application of cryoprotectant blends, the total ash and carbohydrate contents increased significantly compared to those of the control formulation. In surimis, sodium functions by compacting the myofibrillar structure through solubilization, inducing interactions between protein molecules, and ensuring better texture and stability in the final product (Tahergorabi *et al.*, 2012; Yingchutrakul *et al.*, 2022). Its presence in larger quantities increases the mineral concentration. The carbohydrates used in the surimi blends in this study were sorbitol and sucrose, which contributed to the formation of surimi gel; the higher the amount of these agents, the higher the total carbohydrate concentration. Carbohydrates had the greatest influence on the increase in total energy value but also influenced the levels of total lipids and proteins (Table 3).

Color is one of the most important functional properties of surimi and is strongly associated with sensory perception (Sousa *et al.*, 2022). Therefore, several washing cycles of the raw material are used to solubilize pigments such as myoglobin, making the final product as clear as possible for use as an ingredient in the production of various fish derivatives (Park, 2005; Walayat *et al.*, 2022).

When analyzing the colorimetric characteristics together, it can be observed that the actual color of surimi tends towards white, owing to its low color intensities, expressed in different color parameters. Only the lightness (*L**) showed a significant difference among the evaluated treatments, indicating that formulation S1 was the lightest compared to the other treatments. In general, the cryoprotectants used in the different formulations improved water retention compared to surimi without cryoprotectants; this prevents the denaturation of surimi proteins during freezing and thawing, which can affect color(Cao *et al.*, 2016). However, sucrose, used at a concentration of 1% as a cryoprotectant in formulation S1, resulted in a higher moisture content compared to the other formulations, which directly influences the variation of the other macromolecular components, such as protein, lipids and total carbohydrates. These variations in composition also directly influence the reflection of

light, as has been shown in other studies (Hu *et al.*, 2015; Mi *et al.*, 2021). In addition, sodium salts tend to reduce the repulsion between proteins, favoring their binding to each other; these modifications also have a direct influence on the variation of light reflectance (Zhao *et al.*, 2023).

These colorimetric characteristics demonstrated that despite the significant influence of cryoprotectant agents on the lightness of surimi, all surimi evaluated in this study exhibited a high degree of whiteness. This indicates the effectiveness of the washing cycles applied during the process and the potential application of these cryoprotectants in tambaqui surimi for subsequent use as ingredients in surimi derivative production, owing to the versatility of this white-colored product.

The different cryoprotectant blends resulted in a significant increase in the water holding capacity (WHC) of the samples compared to the control formulation (Table 5). This increase is attributed to the effect of the cryoprotectants, since the gel network formed in the CF is weaker than in the other surimis due to the absence of cryoprotectants. Proportionally, these increases were 10.34%, 14.55% and 6.19% for S1, S2 and S3, respectively, compared to CF. The higher proportions of increase in WHC for S1 and S2 is due to the type of cryoprotectants used, which consist of sodium salts, which result in a decrease in repulsion between the proteins molecules, favoring the availability of the protein for interaction with water (Lertwittayanon *et al.*, 2013; Zhao *et al.*, 2023). However, sodium tripolyphosphate was also used for S3, but the presence of sucrose, a cryoprotectant also used in S3, possibly had an antagonistic effect on phosphate, so that only this treatment did not show a significant difference with CF.

Freezing, a stage applied to the samples in this study, also contributes to the formation of the surimi gel network (Walayat *et al.*, 2022). During this process, cryoprotectants interact with the myofibrillar proteins of surimi, controlling the formation of ice crystals and thereby retaining water within the surimi matrix (Park, 2005; Walayat *et al.*, 2022). It is important to note that the WHC values observed in this study exceed those reported for silver carp surimi mixed with starch gums (Mi *et al.*, 2021), and are comparable to those documented for surimi prepared by different washing cycles of tambaqui blood muscle (Bruno *et al.*, 2020) and those stored for 120 days when prepared with matrinxã and different blends of cryoprotectants (Vasconcelos *et al.*, 2016).

The foldability capacity test is a method used to measure the gel quality in surimi. According to Olivares and Castro (2001), C-quality surimi breaks when folded in half but does not separate, A-quality surimi breaks slightly when folded into four parts but remains intact when folded in half, and AA-quality surimi does not break when folded into four parts. Following the same classification, the B-quality surimi breaks slightly when folded in two parts, whereas those of class D break when folded in half and separated into two pieces. Thus, the presence of cryoprotectants contributed to maintaining the structural quality of the proteins and improving their resistance to bending of S1 and S2, and especially S3, all compared with the control formulation.

Correlation between Quality Parameters

The analysis of the arrangement of PCA loadings reveals patterns of correlation among the evaluated parameters, highlighting groupings along the first two principal components (PCs). These groupings reflect the proximity between the parameters, whose positive correlations were confirmed to be statistically significant by Pearson's correlation test. According to Figures 1 and 2, it can be seen that the shear force is centralized at the origin of both PCs, due to the lack of significant correlations with the other nutritional and techno-functional quality parameters of the surimis. However, in the positive quadrant of both PCs, there is a consistent grouping of the colorimetric parameters *C**, *b**, and *a**, justified by the moderate and strong correlations between them.

Although located in different quadrants of PC1, water retention and ashes demonstrate a significant correlation between them. However, while water retention shares the same quadrant with energetic value and carbohydrates, there is no significant correlation with the latter, which, in turn, show a significant positive correlation between themselves. The proximity between proteins, the L* index, moisture, and lipids in the positive PC1 and negative PC2 regions is supported by the moderate and strong positive correlations between them. On the other hand, the fact that the h* parameter is the only one in the negative area of both PCs is due to the fact that this colorimetric attribute shows no correlation with any of the other evaluated parameters.

Pearson correlation combined with PCA were used as a complementary statistical tool to the quantitative evaluation of nutritional and techno-functional parameter data, providing a systematic means of discerning underlying patterns and relationships in data sets from the treatments studied, thus enabling an understanding of the interdependencies between analytes and groups of samples. Therefore, the relationship between the higher average values of moisture, protein and lipids for CF in relation to the other treatments is the reason why these variables stand out as making a significant contribution to the control formulation. This is because CF has no ingredients in its preparation other than washed fish meat, so some of its chemical constituents are concentrated. With the addition of cryoprotectants in the other treatments, these chemical groups are diluted and others are concentrated. For S1, the main contributing variable in the PCA was the total ash content, which is characterized by the presence of the sodium chloride used as a cryoprotective agent in its formulation. S2 and S3, on the other hand, have sorbitol

and sucrose as ingredients in their cryoprotectant blends, which directly influences the significant correlation between these treatments and the carbohydrate and energy value variables. Therefore, the application of multivariate analyses, such as PCA, contributes to a faster visual interpretation of the results in relation to the variables and treatments studied.

Conclusion

Different cryoprotective blends affected the proximal compositions and flexural capacities of the samples in different ways. Through univariate analysis and PCA, treatment S3 stood out as having the best gel quality in terms of flexural strength; however, the samples from treatment S2 showed the best results in terms of protein content, a more satisfactory whiteness profile suitable for processing surimi derivatives, and better water retention capacity. Therefore, the cryoprotective blend used in treatment S2 is the most suitable for use in the production of tambaqui surimi in relation to the final quality of the nutritional and techno-functional characteristics of the surimi gels.

In addition, it is suggested that additional studies be carried out, evaluating the influence of storage time on the quality of tambaqui surimi, as well as the application of other cryoprotective agents with lower caloric value and those obtained from alternative sources, such as proteins, polyols and polymers.

Ethical Statement

Not applicable.

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Author Contribution

First Author: Conceptualization, Investigation, Methodology, Formal Analysis, Visualization and Writing –original, Writing -review and editing; Second Author: Methodology, Visualization and Writing –original, Writing -review and editing; Third Author: Data Curation, Formal Analysis, Writing -review and editing; Fourth Author: Project Administration, Resources, Writing -review and editing; Fifth Author: Data Curation, Formal Analysis, Writing -review and editing; Sixth Author: Project Administration, Resources, Supervision, Writing -review and editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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