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Influence of grilling pretreatment and optimization of *sous vide* processing parameters on the physicochemical and microbiological quality of pirarucu fillet

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Abstract

The demand for high-quality food products has promoted the study of techniques for its processing and conservation. The present research aimed to evaluate the influence of grilling pretreatment on the physical characteristics of pirarucu fillets and the heat transfer process by a computational modelling, and to optimize the *sous vide* process parameters. Before and after the *sous vide* process, the samples were analysed for microbiological, chemical and physical characteristics. There was no significant difference between the total experimental time of grilling and that obtained by computational modelling. Immersion in brine for 300 s in combination with grilling at 200 °C/120 s was selected because of its water-holding capacity (%) 79.40 ± 0.31, texture (N) 1.91 ± 0.40 and value of L^* 74.44 ± 0.38 in the fillets. Cooking at 60 °C for 568.8 s were the best *sous vide* parameters obtained, with highest water-holding capacity (%) 93.60, texture (N) 6.24, ΔE^* 7.43, and with microbiological loads below 6 log CFU/g and 7 log MPN/g in the final product. Useful information obtained from this study highlighted the brine and grill pretreatment in combination with *sous vide* proved it is a potential solution for developing pirarucu products even at an industrial scale.

Keywords

Brine, thermal process, grill, *Arapaima gigas*, vacuum-packaged, *sous vide*, response surface methodology

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INTRODUCTION

The pirarucu (*Arapaima gigas*) is the largest freshwater fish in the world that reaches up 2–3 m and 200 kg. This fish inhabits the Amazon River Basin in South America and it is considered as one of the most important commercial fish species in this region, which has also been well reproduced in captivity (Cortegano et al., 2017). The pirarucu is traditionally preserved and consumed *in natura* or dry-salted without a good standardized processing method that guarantees its quality and preservation (Pino-Hernández et al., 2020). For this reason, it is essential to assess other processing methods that increase the feasibility of introducing the fish into niche markets as a value-added processed product.

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Sous vide cooking technology includes a combination of vacuum packaging, cold shock (after thermal processing), and storage of the products at temperatures below 4 °C for a few hours or days (Baldwin, 2012; Llave et al., 2018). This technology should not be viewed as substitutes for traditional technologies (e.g. brine and grilling), but should be a benchmark for enhancing existing processes and products while improving final product quality (Baldwin, 2012; Kilibarda et al., 2018; Pino-Hernández et al., 2020).

Several authors indicated the viability of using *sous vide* combined with other food processing techniques for different species of fish, leading to excellent results, such as lemon juice (Cosansu et al., 2013), high pressure (Espinosa et al., 2015) and modified atmosphere packing (Hernández et al., 2017). However, it is still necessary to combine the existing techniques to substantially reduce the damage to the physicochemical characteristics in fish products (Llave et al., 2018).

In this context, *sous vide* cooking can be applied in combination with previous brine and grilling pretreatment, but then it is essential to establish heat treatment conditions by evaluating the appropriate heat levels for the thermal process, as well as its effect on texture, water-holding capacity (WHC) and fish fillet colour (Baldwin, 2012; Konno, 2017; Llave et al., 2018).

An adequate heating provides fish fillet with acceptable texture through protein denaturation causing shrinkage of muscle fibres and a more compact tissue texture (Yu et al., 2014). On the contrary, an excessive heating spoils colour, texture and other physicochemical attributes, thus the appropriate degree of heating for the grilling process should be determined (Matsuda et al., 2013; Nakamura et al., 2011; Yu et al., 2014). The influences of the heat transfer and heating type on the fish surface determine colour changes during grilling (Matsuda et al., 2013). Grill pretreatment conditions of 175 to 230 °C are suggested for different types of fish products, allowing the browning to form on the food surface and leading to tastier products that require less cooking time (Llave et al., 2014; Yu et al., 2014).

The effectiveness of the cooking thermal process varies according to the temperature reached and maintained at each point in the food matrix over time, which must be sufficient to inactivate microorganisms and preserve the product (Baldwin, 2012). Therefore, s*ous vide* cooking should receive more attention on potential hazards related to *Clostridium, Staphylococcus* and *Salmonella* spp, in order to maintain the microbiological safety (Hernández et al., 2017; Kilibarda et al., 2018).

To our knowledge, there are no data on the characterization of physicochemical changes of pirarucu heat-treated by the *sous vide* process in combination with previous brine and grilling pretreatment. Therefore, the aims of this study were: (1) to characterize the raw material based on the chemical and microbiological characteristics; (2) to evaluate the influence of grilling pretreatment on the physical characteristics of pirarucu fillets; (3) to characterize by computational modelling the heat transfer during the grilling pretreatment based on the temperature profiles and temperature distributions in the samples; (4) to optimize the *sous vide* process parameters using response surface methodology and desirability function and (5) to control the *sous vide* cooking in terms of temperature histories and microbiological quality in the final product.

MATERIALS AND METHODS

Raw material

The pirarucu samples used in this study were obtained from the industrial production of a fish farming system located in São João de Pirabas, Brazil, where the fish grew in tanks. In this fish farming, the fish were placed in a tank and fasted for 24 h before slaughter, followed by immersion in cold water at 1 ± 1 °C for 1200 s until death. In this work, the fish in life were not manipulated for the authors.

The raw material was stored with ice in an isothermic container and transported to the Fish Products Laboratory of the Federal University of Para (Belem, Brazil). Once the fish were washed and sanitized (5 ppm NaCl solution for 900 s), the dorsal portion was cut, filleted (15 cm × 8 cm × 2.5 cm, length × width × thickness, and weighing approximately 150 g), vacuum-packed and stored at −22 °C until use.

The total weight of the dorsal portion of pirarucu used for this study was 20 kg. A raw material sample of 1 kg was used for the microbiological characterization, thermal conductivity and chemical properties analyses. All experiments were conducted in triplicate.

Microbiological analyses

Microbiological analyses were performed on the raw material and *sous vide*-treated products. The most probable number technique was used to determine the level of coliforms at 45 °C and 35 °C, after incubation for 24–48 h. An indicative analysis was performed for coagulase-positive *Staphylococcus* and *Salmonella* spp (Downes and Ito, 2015). In addition, counts of total aerobic mesophilic and psychrotrophic bacteria were determined by the pour-plate method, using plate count agar (Oxoid Ltd., London, UK) and incubating at 35 °C for 48 h and at 7 °C for 10 days, respectively. The determination of sulphite-reducing *Clostridium* uses as a base medium tryptose sulphite cycloserine agar. Plates were placed in an anaerobic jar and incubated at 36 ± 1 °C for 24 h. All analyses followed the methodology described by Downes and Ito (2015).

Thermal and chemical properties analyses

The thermal conductivity (W/m °C) of the raw material sample was analysed using a KD2 thermal property analyser (Decagon Devices, Inc., Pullman, WA, USA).

The thiobarbituric acid-reactive substances (TBARS) were determined using the methodology proposed by Vieira et al. (2018). The absorbance of the samples was measured at 532 nm. TBARS values were expressed as milligram of malondialdehyde (MDA)eq/kg of sample. The pH value was measured using a digital pH meter (WTW pH 330i Taschen-pH-Meter, WTW GmbH, France). The total volatile bases nitrogen (TVB-N) were analysed according to Cosansu et al. (2013), and the results were expressed as mg N/100 g of sample.

Pretreatments of the pirarucu fillet

As an alternative to improving the technological weaknesses of the conventional *sous vide* process, a pretreatment on fish fillet was evaluated in two steps.

Immersion in a brine solution. Chemical pretreatment was performed on the raw material sample (around 150 g of fillet) by immersion in a brine solution (1:1.5 fish (w):brine (v)) at refrigerating temperature for 300 s, then were removed and left to drain for 2 min. The conditions were defined based on the result of a preliminary experiment (data not shown). The brine solution was prepared as a 3% (w/v) NaCl, and the pH was adjusted with 30% acetic acid to pH 2. The fillets were then grilled as described in Sealing on the grill section.

Sealing on the grill. Thermal pretreatment was performed on a stainless-steel grill (PR-650 L, Progas, Brazil) at 100, 130, 170 and 200 °C. A skewer-type digital thermometer (Incoterm, Brazil) was placed in the centre of the fillet. The time required for the centre of the fillet to reach a stable temperature of 80 °C was considered as the heating time, recorded using a PRO-1 timer (Nautika, Brazil). This procedure was performed on both sides of the fillet. The physical analyses such as texture, WHC and colour were determined for each thermal pretreatment.

Complementarily, simulations of heat transfer were conducted for all the temperatures defined for different grilling pretreatments. During the simulations, measurements were taken every 3 s, considering the variation $\Delta x = \Delta y = \Delta z = 0.005$ cm. This process was followed until the centre of the fillet ($15 \times 8 \times 2.5$ cm, length × width × thickness) reached a stable temperature of 80 °C. The computational model was developed in MATLAB R2019a'' (MATLAB, 2019).

In the heat transfer analysis, the three-dimensional (3D) transient heat conduction of a partial differential equation based on Fourier's law was used equation (1)

$$\frac{\partial T}{\partial t} = \alpha\left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2}\right) \quad (1)$$

where T is temperature (°C); t is time (s); α is thermal diffusivity; x, y and z are the Cartesian coordinates.

The finite difference method (3D) of Geankoplis (1998) was adopted to solve equation (2). Here, the thermal properties of fish are constant in all dimensions. The thermal diffusivity (α) is given by equation (2)

$$\alpha = \frac{K}{\rho Cp} \quad (2)$$

where K is the thermal conductivity (1.07 W/m °C, established in this study); ρ is the density (1067 kg/m^3, established previously by Baldwin (2012)); C_p is the specific heat capacity (3598 J/kg °C, established previously by Orrego (2003)).

On the other hand, to visualize the colour changes in the fillet samples before and after thermal processing, digital images were captured on a digital camera (SX40 HS, Canon, Japan) at the resolution of 1920 × 1080 pixels (simulating the most likely method used in an industrial setting). The images were acquired freehand in a 90° angle perpendicular (60 cm) to the plane where the fillets were located. The lighting system used was standardized; the lamps were positioned in a 45° angle above the fillet. Images were acquired in the JPEG format, additionally, the brightness was evaluated (L^*) using CorelDRAW software (X6-16, Creative Suite, Cowpland Research Labs, Canada) in order to create a colour scale for this parameter.

Physical analyses

Texture analyses (shear strength, SS) were performed on the fillet and cooked samples (2.0 × 5.0 × 5.0 cm, thickness × width × length), using a QTS-25 texture analyser (Brookfield CNS, United Kingdom) equipped with a Warner-Bratzler blade (thickness of 3.0 mm and width of 70 mm), which was applied to the muscle perpendicularly to the muscle fibres. Test conditions were room temperature, load cell of 5 kg, shear strength (in N) and testing speed of 2.0 mm/s. The tests were conducted in six fillets for each treatment.

The WHC of the fillet was determined according to Kato et al. (2016) placing the fillet and cooked samples

between two plates over which a 5-kg weight rested for 5 min. The WHC was calculated using equation (3)

$$WHC(\%) = \frac{mf}{mi} \times 100 \quad (3)$$

where mi = initial mass (g) of the sample; mf = final mass (g) of the sample.

The Commission Internationale de L'Eclairage (CIE) instrumental colour parameters (L^*, a^*, b^*) were recorded using a portable chromameter (Minolta, CR-310, Japan) with a D65 light source. In the CIE $L^*a^*b^*$ colour space, L^* is brightness (varying from 0 = black to 100 = white), a^* varies from green (-a^*) red (+a^*), and b^* varies from blue (-b^*) to yellow (+b^*). Total colour difference/variation (ΔE^*) was calculated using equation (4). The tests were conducted in six fillets for each treatment.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

where ΔL^*, Δa^* and Δb^* are the differences between the colour parameter of the samples processed and the colour parameter of the control sample (unprocessed fillet) $L^* = 63.36 \pm 1.65$, $a^* = -4.07 \pm 0.22$ and $b^* = 7.67 \pm 0.58$.

Preparation of pirarucu *sous vide* product

The raw sample weighing approximately 150 g and with dimensions of 15 cm × 8 cm × 2.5 cm, length × width × thickness, was first pretreated chemically and thermally under the conditions that showed the better physical analyses (Physical analyses section). Next, the pirarucu fillet was vacuum-packed (1:4 fish (w):tucupi sauce (v)), using a packaging machine (F200 Flash, Fastvac, Brazil). Once packaged, the product was pasteurized in a water bath (TE-057, Tecnal, Brazil) at atmospheric pressure using the time and temperature set by the response surface methodology. After *sous vide* processing, the packaged product was cooled in an ice–water solution, with manual stirring until the centre of the fillet reached 0 °C and then stored under refrigeration (1 ± 1 °C).

Optimization of the *sous vide* parameters using response surface methodology

The parameters of time–temperature of the *sous vide* process were defined according to a 2^2 full factorial design in a rotational central composite design (CCD), consisting of four linear trials at two levels (+1 and −1), with axial points −α and +α (−1.41 and +1.41, respectively), and three trials at the centre point as recommended by Kato et al. (2016). The factors used were time and temperature, and the dependent variables determined were characteristics such as WHC (juiciness, as previously described by Kato et al. (2016)), texture (SS) and ΔE^*. High values of WHC, SS and ΔE^* were sought so that the product would maintain its juiciness, texture and colour, according to the initial, full models (equation (5)) the resulting models (response surface models) were found when the WHC, SS and ΔE^* significance levels were $p < 0.05$ or near values

$$Y = f(X1, 2) = \beta_0 + \beta_1(A) + \beta_{11}(A)^2 + \beta_2(B) + \beta_{22}(B)^2 + \beta_{12}(AB) \quad (5)$$

where Y represents the response variable; X_i ($i = 1, 2$) represents the design variables; A is process time (°C); B is process temperature (s). The coefficients of the polynomial equation were represented by β_0 (constant term); β_1 and β_2 (linear coefficients); ${\beta_{11}}^2$ and ${\beta_{22}}^2$ (quadratic coefficients) and β_{12} (interaction coefficients).

Complementarily, the desirability function was applied considering a level of significance of 5% in order to determine the best operational conditions of time–temperature about the responses WHC, SS and ΔE^* for the *sous vide* product.

Control of the *sous vide* process

Two procedures were conducted to evaluate the *sous vide* process performed under the best time–temperature identified: (i) thermal profiles – a skewer-type digital thermometer (Incoterm, Brazil) was placed in the centre of the fillet considered as the slowest heating point, with an acquisition interval of 5 s and data points were then collected in an Excel® worksheet, registering the start and the end of each stage (processing, cooling and reheating) for calculating the pasteurization units and the degree of cooking considering the total thermal process. The reference temperature was set equal to 60 °C and z at 5.5 °C (*Salmonella* spp) for pasteurization units, and equal to 100 °C and z at 33 °C for cook value (Rinaldi et al., 2014); and, (ii) microbiological control – before and after the *sous vide* process the samples were analysed for *Salmonella* spp, sulphite-reducing *Clostridium*, coagulase-positive *Staphylococcus*, coliforms at 35 °C and 45 °C and counts of total aerobic mesophilic and psychrotrophic bacteria.

Statistical analysis

To compare means of the physical variables among experimental thermal treatments, it used a one-way analysis of variance (ANOVA) followed by a post

hoc Tukey's test ($p < 0.05$). The total heating time (i.e. experimental time and time given by three-dimensional transient heat conduction of a partial differential equation based on Fourier's law, as previously described by Geankoplis (1998)) and microbiological data (before and after *sous vide* process) were compared using a Student's *t*-test at a significance level of 5%. Finally, to determine the optimum conditions of time–temperature of the *sous vide* process, the significant effects ($p < 0.05$ or near values) were tested using ANOVA, and also, the response surface to the complete 2^2 factorial design (CCD) as well as the desirability function (obtained from the experimental responses of the physical parameters) were considered. All statistical analyses were undertaken using Statistica software version 5.0 (Statsoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Microbiological and chemical properties of raw material

The results of the microbiological analyses of coliforms at 45 °C of the raw material, *Salmonella* spp. and coagulase-positive *Staphylococcus* were 2.52 log MPN/g, absence in 25 g and 1 log CFU/g, respectively. According to Brasil (2001a) and ICMSF (2011), these values are within the recommended limits for fresh fish coliforms at 45 °C (2 log MPN/g), *Salmonella* spp. (absence) and coagulase-positive *Staphylococcus* (3 log MPN/g).

The chemical properties data of the raw material were pH 5.96 ± 0.03, TVB-N of 2.00 ± 0.03 mg N/100 g and TBARS of 0.40 ± 0.04 mg MDAeq/kg. According to several authors, Brasil (2001b), Özyurt et al. (2009), Hernández et al. (2017) and Vieira et al. (2018) these values are within the recommended limits such as pH 6.0, TVB-N < 30 mg N/100 g and TBARS < 3 mg MDA/kg for fresh fish.

Effects of brine and different time–temperatures of grilling pretreatment on the physical characteristics of the pirarucu fillet

The thermal pretreatment results indicated significant differences ($p < 0.05$) in the WHC, texture and colour (L^*, a^*, b^*) of the pirarucu fillets between treatments (Table 1). The goal of sealing the fillets on a grill was to obtain an 'improved' WHC and texture, and inducing colour variation, and not to cook the product.

In treatment T1, a mass transfer of water (water loss ~28%) from the inner fillet to the heat source was identified, occurring during the first 40 s of the heating process due to the release of free water present in the fillet which is possibly a consequence of the protein denaturation. Kong et al. (2007) showed that this water loss occurs as a result of the reduced interfibrillar volume, caused by shrinkage of the myofilaments.

Comparatively shorter times were used at higher temperatures, producing an increase in WHC, where treatment T4 provided the 'best' results with an average cook loss of around 6.80%. Above 170 °C, the cooking loss percentage was decreased. The changes observed on the surface of the fillet, such as less uniformity, were probably induced by the temperature, which causes protein denaturation (Kong et al., 2007). The actomyosin complex is responsible for the fish's ability to retain muscle water, and the high value of WHC observed in this study indicates good muscle quality (Kong et al., 2007).

From the texture analysis significant differences ($p < 0.05$) were found when comparing all treatments. The treatments T1 and T2 presented the maximum and minimum values of shear strength, respectively, which

Table 1. Experimental results obtained for different conditions of sealing the pirarucu fillets after grilling pretreatment

	Treatments			
	T_1	T_2	T_3	T_4
Parameters	100 °C/330 s	130 °C/240 s	170 °C/150 s	200 °C/120 s
WHC (%)	72.04b ± 0.35	74.41ab ± 0.42	76.46ab ± 0.23	79.40c ± 0.31
SS (N)	2.26 a ± 0.41	1.41b ± 0.48	1.93ab ± 0.45	1.91ab ± 0.40
Colour				
L^*	83.57 a ± 0.23	82.04ab ± 0.22	81.58b ± 0.34	74.44c ± 0.38
a^*	−4.35c ± 0.19	−3.51b ± 0.16	−3.94bc ± 0.26	−1.55 a ± 0.19
b^*	14.58b ± 0.65	14.88b ± 0.31	14.32b ± 0.58	22.24 a ± 0.27

WHC: water-holding capacity; SS: texture.
Results are mean ± standard deviation ($n = 3$). Different letters in the same line indicate statistical significant difference ($p < 0.05$).

indicate these treatments may affect the tenderness of muscle considering this parameter is correlated with the WHC (Table 1). Changes in the texture of pirarucu fillets during the thermal process were similar to the previously reported by Kong et al. (2007) for salmon (*Oncorhynchus gorbuscha*).

On the other hand, when compared the colour parameters, the T1 showed fillets with highest value of lightness (L^*), while T4 resulted in dark-brown fillets associated with lowest value of L^*, which is an evident browning reaction as Maillard due to the temperature of process in the presence of proteins and carbohydrates. Llave et al. (2014) and Yu et al. (2014) found the outer surface appearance typical of grilled food is achieved when the food is at temperatures ranging between 175 and 200 °C as obtained in this study.

The colour parameters a^* and b^* indicated that the fish fillet tended to maintain its redness and yellowness, respectively, and this was attributed to the increased in the pretreatment temperature. Llave et al. (2014) and Matsuda et al. (2013) found that when brightness (L^*) of the sample colour is decreased, the parameters a^* and b^* increased. This behaviour was also observed in the present study. The results of the thermal pretreatment showed that the colour changes on the surface of the pirarucu fillet caused by this pretreatment could be predicted based on water evaporation, together with the increase of the surface temperature and the processing time. Similar results were found by Nakamura et al. (2011), Matsuda et al. (2013) and Kong et al. (2007) when they studied changes in the colour parameters in fish fillet during grilling under different heat transfer systems. They confirmed that the surface browning colour developed is independent of the heat transfer system.

The synergy between the immersion of pirarucu fillet in the brine solution for 300 s, followed by sealing at 200 °C on a grill for 120 s had an important role in preventing discolouration associated with the appearance of white precipitates on the surface of the fillets due to the pretreatment conditions used before the *sous vide* process, which induced to changes in denaturation of protein and gelation and solubilization of collagen. Konno, (2017) and Llave et al. (2018) identified that the effects of the thermal process and the immersion of the fish fillet in a brine solution allow the denaturation of myosin, and actin is affected by salt concentration and process temperature. This fact may be the reason why the white precipitate on the surface of the fillets was not observed. Therefore, among the treatments evaluated, treatment T4 was selected because of its highest WHC, tenderness texture (shear strength) and more attractive colour (browning) on the fillet surface.

Digital images and simulation results of the fillet pretreatment

The colour changes after heating processes were confirmed by comparison with the scale obtained for parameter L^* (Figure 1(a)). These results were similar to those described by Llave et al. (2014) when comparing the digital images and L^* colour scale in fillets of white muscle fish after heating. The digital images acquired for each thermal pretreatment exhibited visual differences at the end of the process, due to the influences of the temperature and process time, ambient temperature, air flow and the heterogeneity of the fish muscle (Figure 1(b)).

The digital images of the pirarucu fillet surface and images of simulations of the heat transfer effect registered the temperature of 25 °C in the fillet sample *in natura*, with a tendency towards bright pink (Figure 1(b) and 1(c)). Conversely, when the surface temperature of the fillet reached 100 °C (Figure 1(b), 1(c) and 1(d)), lightness (L^*) reached its maximum value, possibly due to protein coagulation. Our finding is in agreement with previous observations that protein denaturation causes the fish muscle to become whiter at the beginning of the heating process (Dima et al., 2012).

Subsequently, the treatments at 130, 170 and 200 °C showed a tendency towards moderately darker colours, resulting in the gradual reduction of L^*. This parameter reached a minimum value for the sample treated at 200 °C for 120 s, because of the test temperature, which facilitates rapid water evaporation.

The browning reaction occurs by the rapid evaporation of the water during the first minutes, when a high heating process is applied. It may facilitate the increase of temperature and heat transfer by both conduction and convection. In this study, desirable changes in colour on the fillet surface began when the temperature reached approximately 120 °C (Figure 1(b)). Moreover, at 200 °C for 240 s, a carbonization reaction is estimated to occur on the surface, resulting in undesirable product characteristics. Thus, most changes in the fillet occur within the first 10 minutes of heating haeme proteins (haemoglobin and myoglobin) denaturation make the fish muscle undergo a rapid whitening phase at the beginning of thermal treatment (up to 95 °C) and a slow browning phase at the end (above 150 °C) due to the denaturation of protein and the water evaporating gradually (Kong et al., 2007; Nakamura et al., 2011; Yu et al., 2014).

On the other hand, there was no significant difference ($p < 0.05$) when comparing the total experimental time of heating with that obtained by mathematical modelling (Table 2). The mathematical simulations revealed that rapid thermal conduction occurred in the fish samples. It is initially considered that all of

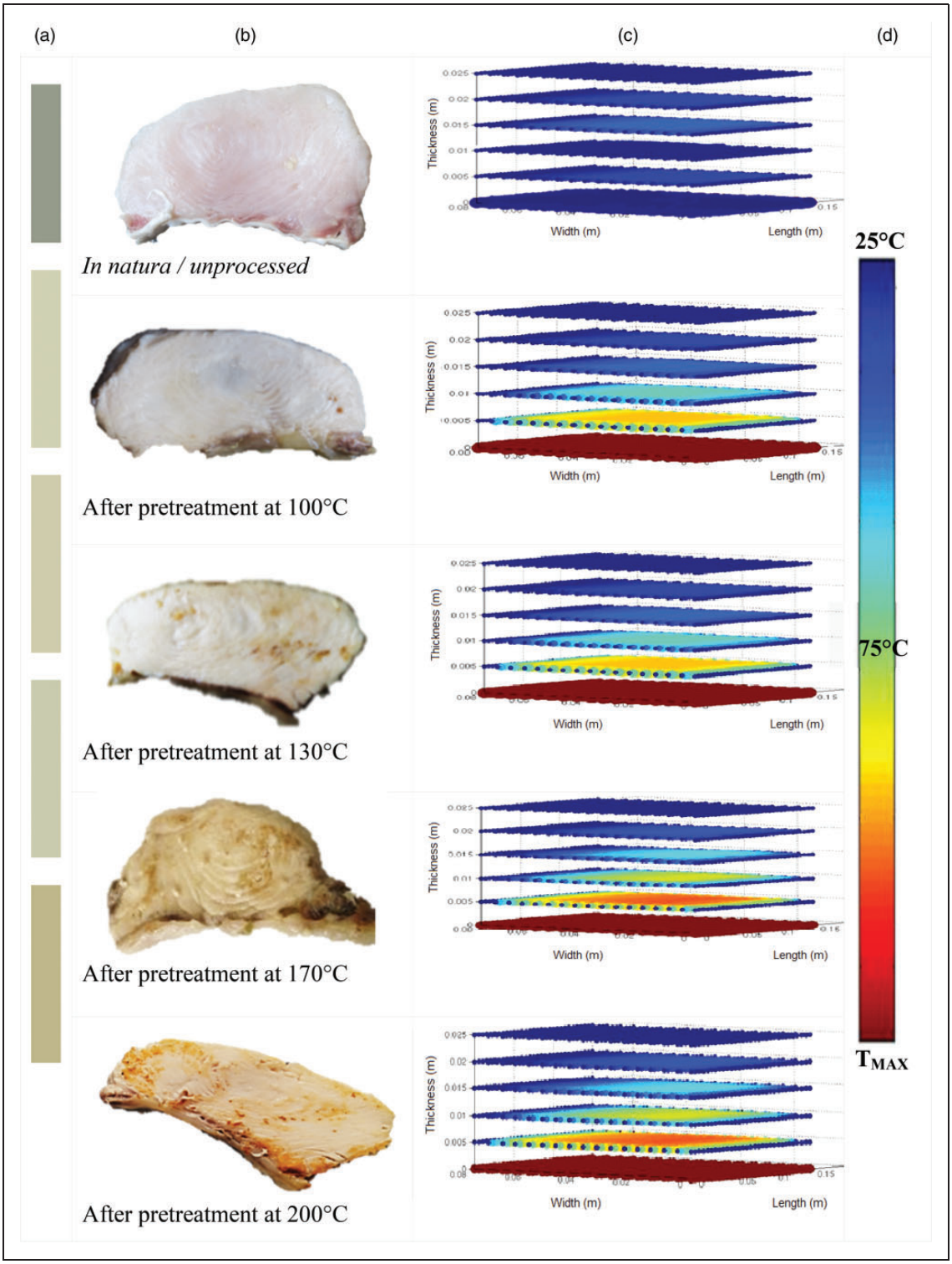

Figure 1. Colour scale for parameter *L** (a); digital images of the pirarucu fillet surface (b); simulation results of the heat transfer effect (c) and temperature scale (d).

the internal points of the fish are at 25 °C, while the surface of the fillet in contact with the heating source reaches the temperature of the grill process immediately (Figure 1(c)).

The thermal processes modelling explicitly described the heat transfer, and accompanied by a colour map, simulated the experimental conditions of the processes. (Figure 1(c)). Similar results were found by Llave et al.

(2014), in which a mathematical approach is used in order to model and predict the expected temperature distribution in fish muscle along the thermal process.

About halfway through each process, the samples were turned, and they showed the same behaviour that appeared in the simulations. This response ensured the formation of a crust on the surface of the fillet when reaching the process temperature, which, in the simulations, is observed by the red colour (Figure 1(c)). As the heating process progressed, the profile colours constantly changed, presenting two main heat flows. The largest temperature gradient was seen on the fillet surface in contact with the hot grill, presenting an upward trend in the centre of the sample, and a little temperature gradient loss in the boundaries of the fillet possibly due to the boundary conditions maintained at lower temperatures (Figure 1(c)).

Table 2. Experimental and modelled times of grill pretreatments for heating to 80 °C at the centre point of the pirarucu fillets

Temperature of the grill (°C)	Initial and target temperature of the centre point of the fillets (°C)	Total heating time (s)	
		t_1	t_2
100	25 to >80	382.8a	330.0a
130		273.0a	240.0a
170		172.2a	150.0a
200		168.0a	120.0a

t_1: time given by the Fourier equation; t_2: experimental time. Different letters in the same line indicate statistical significant difference ($p < 0.05$).

Optimization of *sous vide* parameters using response surface methodology and desirability function

The design matrix (in real and coded forms) and its respective levels are presented along with the experimental values of the WHC, ΔE^* and SS obtained at laboratory scale after the *sous vide* process, in Table 3. The greatest SS (7.65 N), the largest ΔE^* preventing the appearance of white protein precipitates on the fillets (9.61), and the maximum WHC (93.59%) were obtained when the experiments were conducted at 60, 65 and 75 °C, respectively.

The significant linear, quadratic and interaction effects of the full, initial response surface models are shown in Table 4. It is highlighting that the value of F calculated for the lack-of-fit was significant for the variable WHC, while the same parameter for ΔE^* and SS was not significant (Fcal < Ftab), which suggests that the reduced models (simpler) can be used for predictive purposes in the experimental domain studied (Table 4). In addition, the R^2 value was ≥0.83 for ΔE^* and SS parameters studied, which suggests that the model appropriately defined the process behaviour by explaining 83% of the variation in the experimental data. Therefore, it was possible to determine the 'best' condition of the binomial time–temperature of the *sous vide* process.

The ΔE^* and SS parameters depended directly and linearly on time and temperature, respectively (Table 4). Except for the temperature, other parameters had a negative effect. Time had a marked influence on the WHC in the fillet muscle, such that an increase in this factor resulted in a reduction in the WHC, making

Table 3. Levels of the process variables used in the full factorial design (2^2) composed of central and axial points and experimental results for WHC, ΔE^* and SS obtained under different *sous vide* temperature and time conditions

	Coded values		Real values		Means of experimental results		
Assay	Time	Temperature	Time (s)	Temperature (°C)	WHC (%)	ΔE^* (–)	SS (N)
01	−1	−1	600	65	93.59	4.17	7.45
02	1	−1	750	65	92.52	5.71	7.29
03	−1	1	600	85	92.44	5.81	7.40
04	1	1	750	85	92.28	4.39	7.00
05	−1.41	0	568.8	75	84.33	7.68	7.41
06	1.41	0	780.6	75	85.46	9.61	7.47
07	0	−1.41	675	60	89.97	7.35	7.65
08	0	1.41	675	89	92.08	6.79	6.89
09	0	0	675	75	92.19	4.61	7.50
10	0	0	675	75	91.68	2.30	7.20
11	0	0	675	75	92.01	2.80	7.30

WHC: water-holding capacity; ΔE^*: colour variation; SS: texture.

Table 4. Estimated effect and degree of statistical significance for the residual error and pure error for WHC, ΔE^* and SS related to *sous vide* process optimization (full response surface models)

					F value		
Response	Factors	Effect	Standard error	*P* value	Regression	Lack-of-fit	R^2
WHC	Constant	91.010	1.018		218.26	96.405	0.80
	Linear (L)						
	A	−4.593	1.201	**0.001**			
	B	1.428	1.887	**0.029**			
	Quadratic (Q)						
	AA	−1.158	0.747	**0.007**			
	BB	−0.191	1.779	0.499			
	Interaction *AB*	1.317	1.685	**0.027**			
ΔE^*	Constant	3.741	0.666		5.39	1.217^{ns}	0.83
	Linear (L)						
	A	3.389	0.786	**0.044**			
	B	−0.761	1.235	0.580			
	Quadratic (Q)						
	AA	1.456	0.489	0.087			
	BB	3.568	1.165	0.082			
	Interaction *AB*	−0.788	1.103	0.526			
	Model	$3.74 + 1.69$ (*A*)					
SS	Constant	7.317	0.059		6.53	0.279^{ns}	0.87
	Linear (L)						
	A	0.064	0.070	0.563			
	B	−0.548	0.110	**0.064**			
	Quadratic (Q)						
	AA	0.060	0.044	0.087			
	BB	−0.072	0.104	0.654			
	Interaction *AB*	−0.185	0.098	0.526			
	Model	$7.32 - 0.27$ (*B*)					

A: coefficient of the time of the process (°C); *B*: coefficient of the temperature of the process (s); WHC: water-holding capacity; ΔE^*: colour variation; SS: texture; ns: nonsignificant. Regression: F_{tab}: 19.296 (WHC, ΔE^* and SS); Lack-of-fit: F_{tab}: 19.164 (WHC, ΔE^* and SS).

the product less juicy. The *sous vide* process improved the WHC, as this is not lost by exudation and evaporation. The water acts as a plasticizer of muscle proteins, and the water is lost from the myofibrillar structure as a result of protein denaturation, with consequent reductions in the volume of muscle fibres as the cooking temperature is increased. The changes in the arrangement of the myofibrillar structure also affect the light-scattering properties and the perceived whiteness of the fish meat (Yu et al., 2014).

The factor with the greatest positive impact on the ΔE^* was time (*A*), such that an increase in time caused a higher tendency to browning, which is important for the product appearance. Colour variation during thermal processes involves four steps: (i) protein denaturation; (ii) water evaporation; (iii) the browning reaction and (iv) the carbonization reaction (Llave et al., 2014). In our study, the L^* parameter was the best indicator of the colour changes of the fillet surface when compared with a^* and b^*, these colour variations are most apparent with the increasing of temperature.

The temperature (*B*) negatively affected the muscle SS in pirarucu fillet, which is not a favourable result for product quality. This pattern was also observed in a previous study, wherein a linear decrease in fish muscle texture started at 60 °C (Kato et al., 2016).

The response surface and its respective contour lines generated by the models proposed for ΔE^* and the SS response are shown in Figure 2(a) and 2(b), respectively. Through the response surfaces and level curves, it was evident that the ΔE^* of the *sous vide* depended directly and linearly on time, while the SS depended directly on temperature.

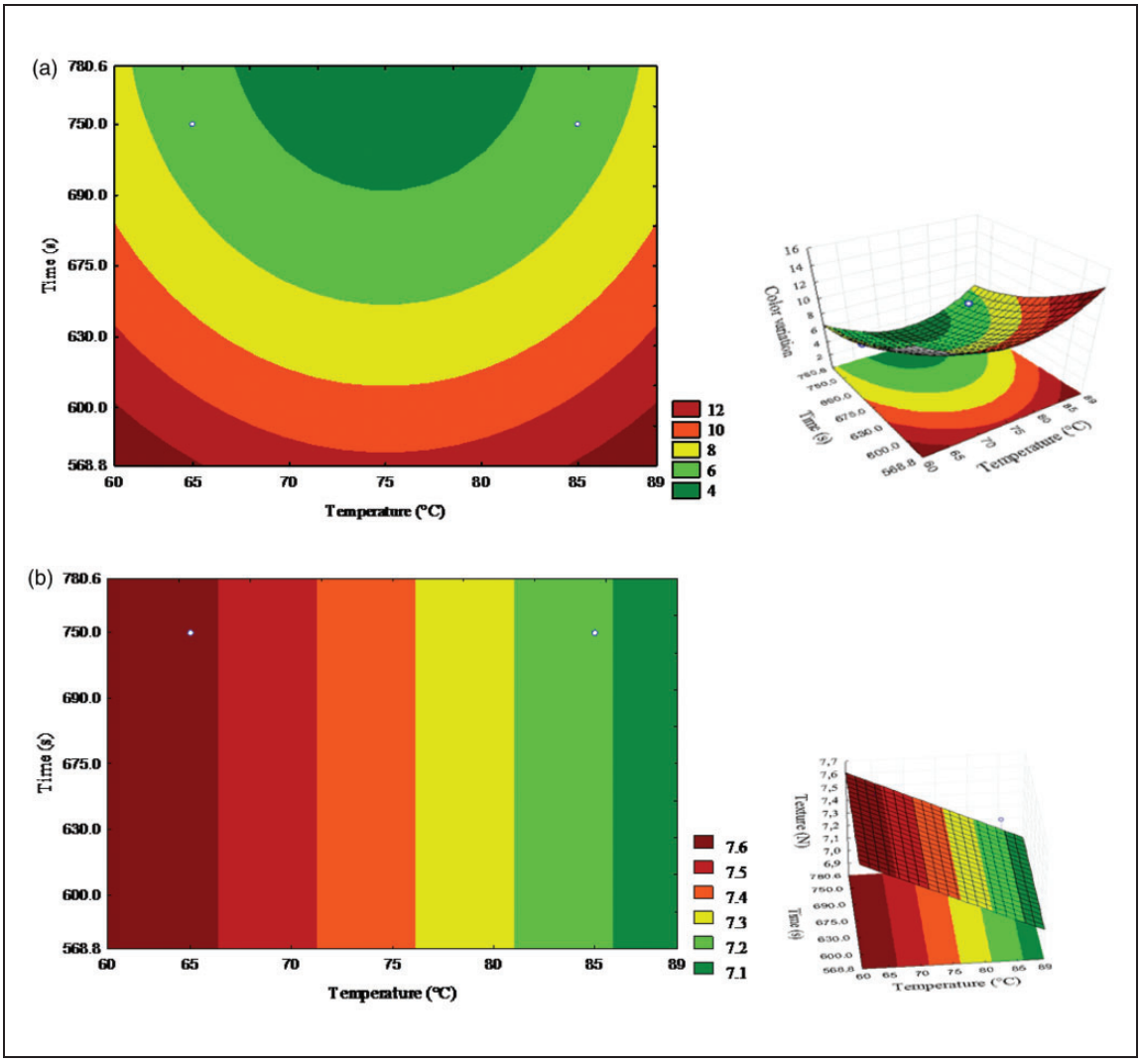

Figure 2. Contour lines and response surface showing the effect of colour variation (a) and texture (b).

The results obtained with the CCD design and through the desirability function showed that it was possible to set the binomial time–temperature conditions of the pirarucu *sous vide* in the evaluated experimental area (Figure 3). In this figure, the trends revealed by the time–temperature factors are represented by the horizontal blue dashed lines (the top three graphs), which register the average value of each dependent variable. The intersection of the red lines in the corresponding graphs indicates the graph maximum for the variables WHC, SS and ΔE^*, corresponding to the values 93.60, 6.24 and 7.43, respectively.

Based on the response surface graphs and the desirability function (Figure 3), the optimized operating conditions can be set for the development of pirarucu fillet *sous vide* in the evaluated experimental area, and the optimum time–temperature conditions used at the laboratory scale are 60 °C and 568.8 s, with a desirability value of 0.80. This temperature is similar to that reported by Kato et al. (2016) and Cosansu et al. (2013). According to the current investigation, the use of temperatures between 65 and 95 °C for 600 to 3600 s, with rapid cooling to reduce the temperature of the centre of the product to 0 °C, is appropriate condition for *sous vide* processing of fish.

Control of the best binomial time–temperature condition for *sous vide* processing

Thermal profile. The control of the *sous vide* process registers the temperatures and times of each step of heating, processing, cooling and reheating of the *sous vide* pirarucu (Figure 4). In the first stage, the red line specifies the initial and final temperatures in the centre of the fillet of 25 and 60 °C, respectively, which were reached at 0 and 270 s. This step weakly contributed to

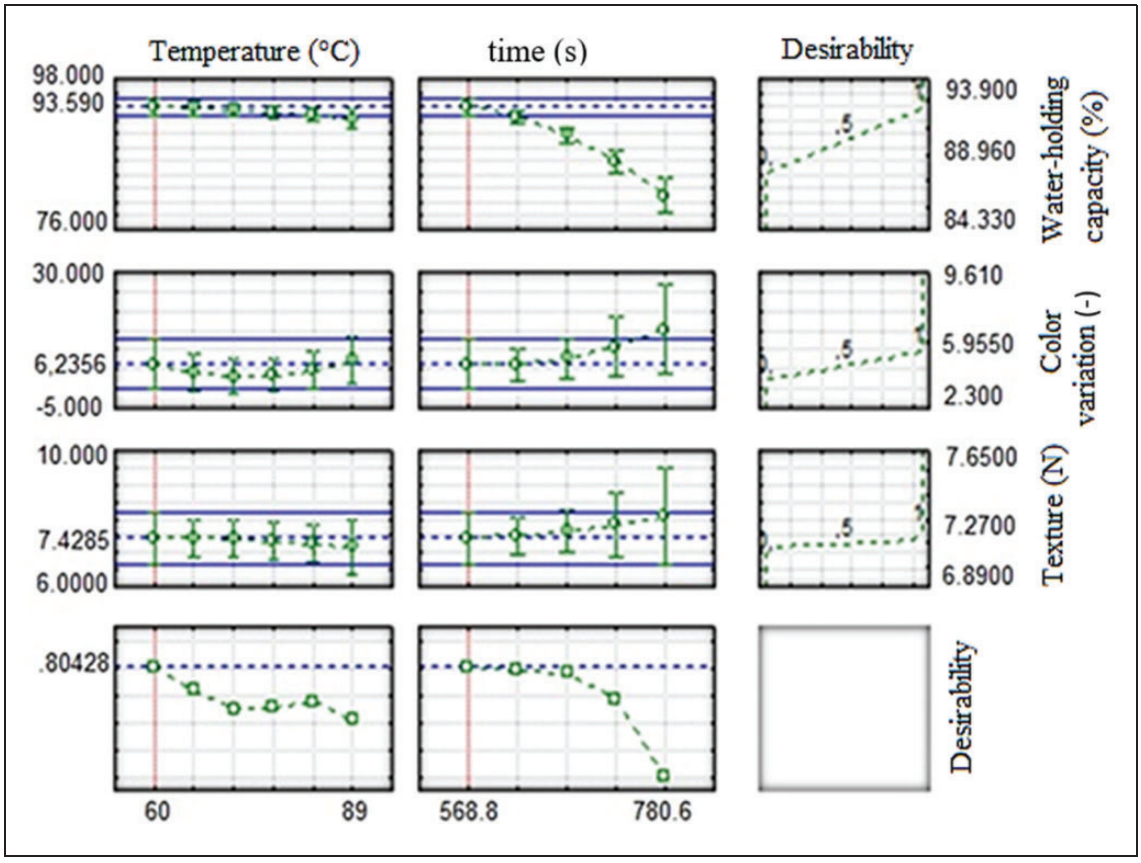

Figure 3. Profile of the predicted/optimized values and desirability function of the variables applied to the *sous vide* process.

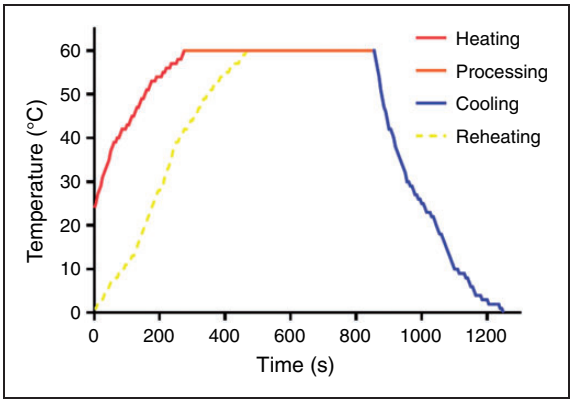

Figure 4. Thermal profiles of the best *sous vide* process.

the total reduction of microorganisms, including *Salmonella* spp., coliforms at 45 °C and coagulase-positive *Staphylococcus*.

The highest influence on the lethality to the pathogenic microorganism of reference (*Salmonella* spp.) occurred in the second stage (orange line), when the centre of the product reached 60 °C and remained at this temperature for 568.8 s. According to Baldwin (2012), the condition of 60 °C and 328.8 s is sufficient to reduce *Salmonella* spp., to ensure microbiological safety and quality. When conditions lead to the absence of *Salmonella* spp. in a vacuum-packed product, a reduction of coliforms at 45 °C and coagulase-positive *Staphylococcus* is implied. The third stage (blue line) registered a rapid loss of heat from 60 to 50 °C for 60 s. The temperature of 0 °C was reached in about 390 s in an ice bath with manual stirring. Immediately after cooling, the product was stored under refrigeration (1 °C).

Finally, after storage, the *sous vide* fillet needs reheating (yellow discontinued line) before consumption. The appropriate time–temperature condition for this stage was established as 480 s following immersion of the fillet in the water bath at 60 °C.

Overall, the product does not represent a microbiological potential risk for consumers. Regarding the pasteurization and cooking values calculated from experimental time–temperature profiles, 613.32 and 46.53, respectively, achieved a sufficient inactivation of pathogens from pirarucu fillet for the best binomial time–temperature condition, ensuring the microbiological safety and quality attribute.

Table 5. Microbiological analyses before and immediately after processing the *sous vide* product

Microorganism	Values	
	Before processing the *sous vide* product	After processing the *sous vide* product
Salmonella spp.	Absence in 25 g	Absence in 25 g
Coagulase positive *Staphylococcus*	2.00 a log CFU/g	0.41 b log CFU/g
Coliforms at 35 °C	2.36 log MPN/g	–
Coliforms at 45 °C	2.63 a log MPN/g	0.32 b log MPN/g
Sulphite-reducing *Clostridium*	Absence	Absence
Mesophilic bacteria	3.40 a log CFU/g	1.70 b log CFU/g
Psycrotrophic bacteria	2.40 log CFU/g	–

MPN: most probable number; CFU: colony forming units; ''–'': not performed.
Different letters in the same line indicate statistical significant difference ($p < 0.05$).

Microbiological control. The microbiological analyses before and after the *sous vide* processing (Table 5) verified the absence of *Salmonella* spp. and sulphite-reducing *Clostridium*, and low counts of coagulase-positive *Staphylococcus* (2.00 log CFU/g) and coliforms at 45 °C (2.63 log MPN/g). These values were within the standards set by Brazilian legislation IN 12/2001 (Brasil, 2001a) and ICMSF (2011).

The mesophilic and psychrotrophic microorganism counts in the samples before *sous vide* processing were 3.40 and 2.40 log CFU/g, respectively, which decreased (1.70 log CFU/g) after *sous vide* processing performed under optimized conditions. The coliform results at 35 °C also decreased from an initial value of 2.36 log CFU/g in the raw material to 0.32 log CFU/g after *sous vide* processing. The microbiological analyses confirm that before and after the *sous vide* processing, hygienic and sanitary procedures were properly followed. In conclusion, the microbial load did not reach the maximum limits of 6 log CFU/g and 7 log MPN/g established by Brazilian legislation (Brasil, 2001a) and the ICMSF (2011), respectively.

CONCLUSION

The analysis of pretreatments, such as those assessed in this study for *sous vide* cooking, is essential to evaluate the physical characteristics of the cooked products and ensure food safety. Brining combined with the grilling pretreatment (at 200 °C for 120 s) of pirarucu fillet had a synergistic effect by solving technological difficulties in the conventional process such as the appearance of white precipitates caused by denaturing proteins. The computational modelling processes corresponded with the experimental conditions of the heat pretreatment. This 3D model also showed how the heat transfer in the fillet evolved. Texture and colour variation were significantly affected by the choice of the best binomial time–temperature of the *sous vide* process. Cooking at 60 °C for 568.8 s was the 'best' processing condition identified for developing the *sous vide* product according to the response surface methodology and the desirability. Based on this result, it was possible to explain changes in colour, WHC and texture, and to guarantee the food safety of the cooked products. Therefore, the development of pirarucu *sous vide* is an excellent option for developing a fish value-added product to be supplied to market niches.

DECLARATION OF CONFLICTING INTERESTS

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