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Chilled Pacu (*Piaractus mesopotamicus*) fillets: Modeling *Pseudomonas* spp. and psychrotrophic bacteria growth and monitoring spoilage indicators by ^{1}H NMR and GC–MS during storage

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ABSTRACT

This study aimed to assess the growth of *Pseudomonas* spp. and psychrotrophic bacteria in chilled Pacu (*Piaractus mesopotamicus*), a native South American fish, stored under chilling conditions (0 to 10 °C) through the use of predictive models under isothermal and non-isothermal conditions. Growth kinetic parameters, maximum growth rate (μ_{max}, 1/h), lag time (t_{Lag}, h), and (N_{max}, Log_{10} CFU/g) were estimated using the Baranyi and Roberts microbial growth model. Both kinetic parameters, growth rate and lag time, were significantly influenced by temperature ($P < 0.05$). The square root secondary model was used to describe the bacteria growth as a function of temperature. Secondary models, $\sqrt{\mu} = 0.016\ (T + 10.13)$ and $\sqrt{\mu} = 0.017\ (T + 9.91)$ presented a linear correlation with R^2 values >0.97 and were further validated under non-isothermal conditions. The model's performance was considered acceptable to predict the growth of *Pseudomonas* spp. and psychrotrophic bacteria in refrigerated Pacu fillets with bias and accuracy factors between 1.24 and 1.49 (fail-safe) and 1.45–1.49, respectively. Fish biomarkers and spoilage indicators were assessed during storage at 0, 4, and 10 °C. Volatile organic compounds, VOCs (1-hexanol, nonanal, octenol, and indicators 2-ethyl-1-hexanol) showed different behavior with storage time ($P > 0.05$). ^{1}H NMR analysis confirmed increased enzymatic and microbial activity in Pacu fillets stored at 10 °C compared to 0 °C. The developed and validated models obtained in this study can be used as a tool for decision-making on the shelf-life and quality of refrigerated Pacu fillets stored under dynamic conditions from 0 to 10 °C.

1. Introduction

Pacu (*Piaractus mesopotamicus*) is a neotropical freshwater fish native to the Brazilian fauna. This species is widely found in the *Bacia do Prata* region, mainly in the Central-West and South and Southeast Brazil (Calcagnotto and DeSalle, 2009). Pacu is one of the most consumed freshwater fishes in Brazil due to several factors such as i) easy handling and breeding, ii) rapid growth, iii) easy adaptation to artificial feeding, iv) resistance to low temperatures, v) high acceptability due its pleasant flavor (Urbinati and Gonçalves, 2005). Besides, Pacu stands out in fish farming as it is well-appreciated by the population due to the characteristics of the meat (firm and delicious), resulting in quite profitable sales (Urbinati and Gonçalves, 2005). Despite the above, Pacu meat has high pH (6.5–6.8), moisture (60–80 %), and lipids (8–13 %) (Tanamati

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et al., 2009; Zuanazzi, 2013), and as other fish matrices are prone to the growth of a wide diversity of microorganisms which will result in its spoilage (Anagnostopoulos et al., 2022; Doulgeraki et al., 2010; Doyle et al., 2003; Zhuang et al., 2020). As such, intervention strategies have commonly been applied to preserve and extend the shelf-life of fish (Baptista et al., 2020; Odeyemi et al., 2018a, b; Tsironi et al., 2020).

Cold chain (ice and freezing) is the primary preservation approach used for fishes. After fishing, ice is frequently employed until fish reach processing plants that use chilling and ice throughout the storage and commercialization (chilled storage). Low temperatures reduce the metabolic activity of the spoilage microorganisms and typically extend the shelf-life of the fish (Gram and Dalgaard, 2002). Despite this, variations in the temperature during cold chain may occur, and these failures are often related to abuse of temperature during transportation between retail and final consumer (Gill et al., 1997; James et al., 2006; Raab et al., 2008; Rediers et al., 2009). Nonetheless, temperature fluctuations may also occur as a result of the thermal properties of the food, variations in the environmental conditions, packaging, and the initial product temperature. As a result, these factors may result in microbial growth further limiting the product's shelf life (Anagnostopoulos et al., 2022; Zhuang et al., 2020).

Fish spoilage is mainly caused by the growth of psychotropic bacteria, with the predominance of *Pseudomonas* spp., which can still grow at temperatures below 4–7 °C. Additionally, *Photobacterium* spp., *Shewanella* spp., and lactic acid bacteria are also related to fish spoilage, depending on packaging and storage conditions (Anagnostopoulos et al., 2022; Zhuang et al., 2020). The production of metabolites and off-flavors, such as amines, sulfides, alcohols, aldehydes, ketones, or organic acids, that are associated with fish spoilage is highly associated with these microorganisms (Dalgaard et al., 2006; Gram and Dalgaard, 2002). Hence, measuring metabolic by-products can be a good predictor of fish quality.

Given the above, the estimation of the kinetic growth parameters of psychotropic bacteria and *Pseudomonas* spp. during cold chain is critical for building models capable of predicting their behavior on fluctuating temperature conditions during transportation and commercialization of aerobically packed fish. The kinetic growth parameters of foodborne microorganisms have been estimated using mathematical models (Baranyi and Roberts, 1994; Baranyi et al., 1995; Possas et al., 2022). Predictive models have been developed and employed to estimate kinetic growth parameters of foodborne bacteria in several food matrixes (Tenenhaus-Aziza and Ellouze, 2015), contributing to evaluating different time/temperature exposure scenarios impacting the product's shelf-life. Despite this, the predictive modeling of the impact of storage conditions and assessment of the shelf-life of freshwater fishes, particularly of native fishes from South America, is scarce (Pino Hernández and Joele, 2017; Rodrigues et al., 2017; Yu et al., 2016). Thus, as predictive models comprise a milestone for quick and scientific-based decisions regarding the shelf-life and quality of fishes and other foods, in this study the growth kinetic parameters of *Pseudomonas* spp. and psychrotrophic bacteria were estimated using a predictive modeling approach in chilled Pacu (*Piaractus mesopotamicus*) stored at different conditions. Furthermore, multiple indicators of fish spoilage during chilling conditions of storage were also investigated.

2. Materials and methods

2.1. Fish slaughtering, filleting, packing, and storage conditions

Ungutted fresh Pacu (*Piaractus mesopotamicus*) samples (n = 20) were collected in different seasons (February/December and June) from a fish farm in the state of São Paulo, Brazil. The slaughter was carried out by hypothermia, which causes brain destruction, following the protocol of animal ethics, minimizing pain and distress in the fish. Fish samples were washed, gutted, filleted, and transported to the laboratory on ice within 4 h. Three fillets of 20 g each (per sample) were placed in individual absorbent trays, totaling about 60 g, and wrapped with PVC films. The packaged fillets were stored under controlled isothermal conditions (0, 2, 4, 6, and 10 °C), which comprises the range of ideal storage conditions to abuse conditions fishes can be exposed during commercialization. Total psychrotrophic bacteria and *Pseudomonas* spp. growth was monitored at regular intervals. For each storage temperature studied, at least 15 sampling points were obtained. At each sampling point, triplicate samples were collected and employed for microbiological, ^{1}H NMR, and CG analyses.

2.2. Microbiological analysis

A total of 10 g of each sample was aseptically cut into small pieces and transferred to a stomacher bag containing 90 mL of sterile buffered peptone water (BPW) (Himedia, Mumbai, India) (1:10 (v/v) dilution). The samples were then homogenized in a Stomacher 400 (Model BA7021, Seward, London, England) for 1 min. Serial dilutions (1:10 v/v) were prepared, and 0.1 mL were spread in duplicate in Plate Count Agar (PCA) (Kasci, Roseto degli Abruzzi, Italy) with 0.5 % NaCl (Dinâmica, São Paulo, Brazil) and *Pseudomonas* Agar Base (PSA) (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). PCA with 0.5 % NaCl and PSA agar plates were used to enumerate psychotropic bacteria and *Pseudomonas* spp., respectively. PCA and PSA plates were incubated at 7 ± 1 °C for 10 days and at 25 ± 1 °C for 48 ± 2 h, respectively. Confirmation of *Pseudomonas* spp. was performed for up to 5 colonies per plate using the Oxidase test (Laborclin, Rio Grande do Sul, Brazil) and Kligler Iron Agar test (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). The results were expressed as $\log_{10}$ CFU/g.

2.3. Predictive model development

A typical two-step modeling approach, including primary and secondary modeling, was employed to quantify the effect of temperature on the kinetic parameters of *Pseudomonas* spp. and psychrotrophic bacteria.

2.3.1. Primary modeling

Microbial growth data were collected at different temperatures (0, 2, 4, 6, and 10 °C). For each temperature, kinetic parameters, maximum population (N_{max}, $\log_{10}$ CFU/g), maximum growth rate ($\mu_{max,\ 1/h}$), and lag time ($t_{Lag,\ h}$), were calculated by fitting the experimental data to the primary model of Baranyi and Roberts (1994), using DMFit version 3.5 Excel® add-in.

2.3.2. Secondary modeling

The square root model proposed by Ratkowsky et al. (1982) was employed to describe the effect of temperature on the growth rate of bacterial growth (Eq. 1).

$$\sqrt{\mu_{max}} = b(T - T_0) \quad (1)$$

where: μ_{max}: maximum growth rate (1/h); T temperature; T_{min}: theoretical minimum temperature; b: slope of the regression line.

2.3.3. Model validation under dynamic temperature conditions

Two scenarios were used to assess the predictive capability of the secondary model of *Pseudomonas* spp. and psychrotrophic bacteria growth, mimicking fish storage under non-isothermal conditions (temperature fluctuation).

Pacu fillet samples were stored under non-isothermal conditions to simulate possible time and temperature abuse that may occur during storage and transportation. The samples were prepared as described in Section 2.1 following refrigerated storage in two different scenarios. In the first scenario, the Pacu fillet samples were stored twice at the following temperature sequence: 6 °C, 0 °C, 4 °C, 2 °C, and 10 °C, remaining 12 h at each temperature. In the second scenario, the Pacu fillet samples were stored at the same temperature regime (6 °C, 0 °C,

4 °C, 2 °C, and 10 °C), remaining 24 h at each temperature until the end of the shelf-life. During the experiments, the temperature of Pacu fillet samples was measured at each sampling point. All these experiments were repeated twice. Counts of *Pseudomonas* spp. and psychotrophic bacteria were performed as described in Section 2.2.

The predictive growth models under non-isothermal conditions were developed by a set of differential equations (EDOs) as previously described (Baranyi et al., 1995). These EDOs were replaced in the equation described by Ratkowsky et al. (1982) for growth rate determination. The fourth-order Runge-Kutta method was applied to solve the EDOs using the SCILAB software (Scilab Enterprises, 2012, version 6.0.1). This approach allowed the estimation of the population of each microorganism under non-isothermal conditions.

2.3.4. Model validation

Observed and predicted growth rate (μ_{max}) responses were compared using the bias, B_f, and accuracy A_f factors (Ross, 1996).

2.4. Gas chromatography-mass spectrometry (GC–MS) analysis

2.4.1. Samples preparation and chromatographic analyses

The volatile compounds were extracted according to Xu et al. (2015). After each sampling point, the samples for CG analysis were immediately stored at −80 °C to prevent the loss of analytes. Prior to the analysis, each sample was kept at room temperature (~22 °C) for 30 min. The samples (3 g) were then transferred to 40 mL vials, where 6 mL of sodium chloride (0.36 g/mL) were added. Next, the vials were sealed with polyethylene and silicone septum cap and kept at 50 °C for 15 min to let the sample and headspace equilibrate before SPME analysis. Headspace was exposed to the SPME fiber for 15 min at 50 °C and immediately desorbed in a gas chromatographer injector at 250 °C.

Chromatographic analyses were performed in an Agilent 7890A linked to an Agilent 7000 triple quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA). Helium was employed with a flow rate of 1 mL/min. Data were acquired by using the Agilent MassHunter Workstation Software Qualitative and Quantitative Analysis for QQQ (Agilent Technologies Inc.). National Institute of Standards and Technology (Software Mass Spectral Library Version 2.0, 2011, Gaithersburg, Maryland, USA) MS database was employed for ion fragmentation consultation. All analyses were performed in splitless mode, using a liner suitable for SPME analysis. HP-5 ms capillary column 30 m × 0.25 mm ID × 1.0 μm d_f (Agilent Technologies, Palo Alto, CA) was employed. Injector and oven temperature parameters were optimized according to Xu et al. (2015). After extraction, desorption was performed immediately on the injector in the GC injector at 250 °C. The column temperature ramp was 40 °C for 1 min, then raised to 100 °C at 4 °C/min, and elevated to 230 °C at 6 °C/min, which was the final temperature held for 1 min.

Mass spectra were acquired using electron ionization (70 eV) ion fragmentation. Nitrogen and Helium were employed as collision gas, followed by data acquisition at Multiple Reaction Monitoring (MRM). The full scan was acquired prior to MRM analysis using the following conditions: 10000 amu/s scan speed and 30–400 *m/z* mass range to investigate product ions and all relevant mass transitions (Table 1).

2.4.2. Biomarkers determination

The biomarkers were chosen according to the concentration and formation throughout the storage period at 0 °C, 4 °C, and 10 °C in three distinct points (initial, intermediate, and final). A full scan analysis was conducted, and the fish biomarkers were selected based on the significant quantification area. Mass transitions (*m/z*) for the four elected biomarkers are shown in Table 1.

Table 1
Mass transitions (*m/z*) and respective collision energies (V) acquired on Multiple Reaction Monitoring (MRM) mode for the elected biomarkers.

Compounds	Retention time (min)	Transition 1		Transition 2	
		m/z	Collision energy (V)	*m/z*	Collision energy (V)
1-Hexanol	12.497	56.0 → 41	5	68.9 → 41	5
Nonanal	13.538	56.9 → 41	5	69.9 → 41	5
Octenol	15.474	57.0 → 40	15	69.9 → 42	5
1-Hexanol, 2-ethyl	16.644	57.0 → 41	5	69.9 → 55	5

2.5. ^{1}H NMR analysis

2.5.1. Sample preparation

For ^{1}H NMR analysis, the samples were immediately stored at −18 °C. After freezing and before 1H NMR analysis, the samples were lyophilized. For lyophilization, the samples were placed in sterile Petri dishes and covered with PVC film containing small holes to allow air circulation. The samples were returned to the freezer (−18 °C), and on the next day, they were transferred to the freeze dryer (Terroni, LS3000, São Paulo, Brazil).

2.5.2. Monitoring the metabolites by ^{1}H NMR spectroscopy and molecular identification

The ^{1}H NMR analysis procedures were performed as described in Baptista et al. (2020). Fish samples (100 mg) were directly mixed with 800 μL of a stock solution of buffered deuterate water (D_2O) (99.8 %) containing 56.16 mg/mL of disodium phosphate (Na_2HPO_4) and 48.17 mg/mL of NAH_2PO_4 and 0.13 mg/mL of trimethylsilyl propanoic acid (TMSP-d_4) (internal standard to δ 0.0). The mixture was stirred at 1.500 rpm for 3 min at room temperature and then centrifuged at 806.4 *g* for 5 min. The supernatant was collected into 5 mm NMR tubes. This procedure was performed in quadruplicate for each storage temperature (0 °C and 10 °C).

The ^{1}H NMR experiments were performed on an Agilent 600-MHz spectrometer with a 5 mm (H-F/^{15}N–^{31}P) inverse detection One Probe™ with an actively shielded *Z*-gradient. The ^{1}H NMR spectra were acquired in triplicate under quantitative conditions using the PRESAT pulse sequence for water suppression at δ 4.86, calibrated pulse to 90° (9.62 s pulse length at 58 dB of power), 32 scans, 64 k of time domain points with a spectral window of 20.0 ppm, acquisition time of 5.0 s, relaxation delay of 25.0 s. A pre-fixed value of 36 for receiver gain was used for all the acquisitions, and the temperature was controlled to 298 K. The spectra were processed by applying exponential multiplication of the FIDs by a factor of 0.3 Hz and Fourier transformation of 64 k points. Phase correction was manually performed, and the baseline correction was applied over the entire spectral range (Alves Filho et al., 2017).

The sample compounds were identified through the 2D-NMR experiments of COSY (correlation spectroscopy), HSQC (heteronuclear single quantum coherence), and HMBC (heteronuclear multiple bond correlation) by comparison using an open access database (*www.hmdb.ca*) and literature reports (Alves Filho et al., 2016; Cai et al., 2014; Lin et al., 2017; Shumilina et al., 2016; Wagner et al., 2014; Wei et al., 2017). The molecular structures, ^{1}H and ^{13}C chemical shifts, multiplicity, correlations and constant coupling, 2D-NMR data acquisition and processing are available in the Supporting Information.

The compounds that presented high variations in chemometrics and did not exhibit overlapping resonances were quantified using the external reference method provided by the VnmJ™ program (version 4.2, Agilent). The quantitative results were evaluated using the ANOVA single factor. The Origin™ 9.4 software was applied for the statistical analysis at the significance level of 0.05. Tukey's test was used for mean

comparison and Levene's test for variance homogeneity. The combined uncertainties were based on the analytical errors, replicates of the sampling, and standard deviation of the triplicate of ^{1}H spectra acquisitions (Alves Filho et al., 2017).

2.5.3. Chemometric analysis

Chemometric analysis was performed to investigate the variability of the composition of the fish samples stored under temperatures of 0 °C and 10 °C during two different batches (February/December and June). The ^{1}H NMR spectra (acquired in triplicate for each sample replicate - totaling 24 ^{1}H NMR spectra) were converted to American Standard Code for Information Interchange (ASCII) format to matrix construction, which was exported for chemometric analysis using The Unscrambler X™ program 10.4 (CAMO software, Woodbridge, NJ, USA). The Singular Value Decomposition (SVD) algorithm was used to decompose the matrix (24 samples × 6947 variables = 166,728 NMR data) to reduce data dimension and to observe trends of the sample variability with a confidence level of 95 % (Hotelling, 1933).

To overcome problems related to signal shifts (misalignment tends) due to minor variations in pH and temperature, the compounds' signals commonly present in the spectra were assigned using the COW (Correlation Optimized Warping) method, even after the TMSP-d_4 calibration. PCA was carried out after the mean-centered processing over the samples and baseline correction with normalization over the variables since these pretreatments enhanced the differences between the sample compositions (Sucupira et al., 2017).

3. Results and discussion

3.1. Predictive modeling of Pseudomonas spp. and psychrotrophic bacteria

The present study aimed to develop and validate models to predict the growth of *Pseudomonas* spp. and psychrotrophic bacteria in refrigerated Pacu fillets at temperatures from 0 to 10 °C. This temperature range was chosen to simulate conditions that the Pacu fillets may be exposed to during commercialization, from ideal to abuse. *Pseudomonas* spp. was chosen for monitoring because it is an essential and well-known genus linked to the spoilage of foods of animal origin, which contain significant amounts of protein and fats (Anagnostopoulos et al., 2022; Zhuang et al., 2020). Besides, *Pseudomonas* spp. is one of the principal genera belonging to the group of psychrotrophic bacteria, and as such, tends to be present in high abundance in fish and seafood (Koutsoumanis et al., 2006; Koutsoumanis and Nychas, 1999, Mikš-Krajnik et al., 2016, Odeyemi et al., 2018a, b; Parlapani, 2021; Parlapani et al., 2014). Besides, in this study, psychrotrophic bacteria were also monitored as this microbial group is an indicator of the total bacterial population in chilled foods and their counts are often associated with the reduction of the product's shelf life (Anagnostopoulos et al., 2022; Zhuang et al., 2020).

Growth data of *Pseudomonas* spp. and psychrotrophic bacteria were collected at different refrigerated isothermal conditions (0, 2, 4, 6, and 10 °C) (Fig. 1). The data was fitted to the Baranyi model, and the kinetic parameters maximum growth rate ($\mu_{max,\ 1/}$h), lag time ($t_{Lag,}$ h), and maximum population ($N_{max,}$ Log CFU/g) were estimated (Table 2). The determination coefficient (R^2) values were > 0.96 for all fitted curves at isothermal temperatures. *Pseudomonas* spp. and psychotrophic bacteria showed a similar bacterial growth profile, with an initial population of 3.6 ± 0.4 log CFU/g and 3.9 ± 0.4 log CFU/g, respectively (Fig. 1). Both microbial groups reached a maximum population density (N_{max}) of 9.8 ± 0.3 log CFU/g at all studied temperatures.

The temperature increase resulted in an increase in the growth rates and a reduction in lag time duration. At 10 °C, both *Pseudomonas* spp. and psychrotrophic bacteria grew nearly seven times faster than at 0 °C (Table 2). Increasing the storage temperature from 2 °C to 4 °C resulted in significant ($P < 0.05$) differences between *Pseudomonas* growth rates.

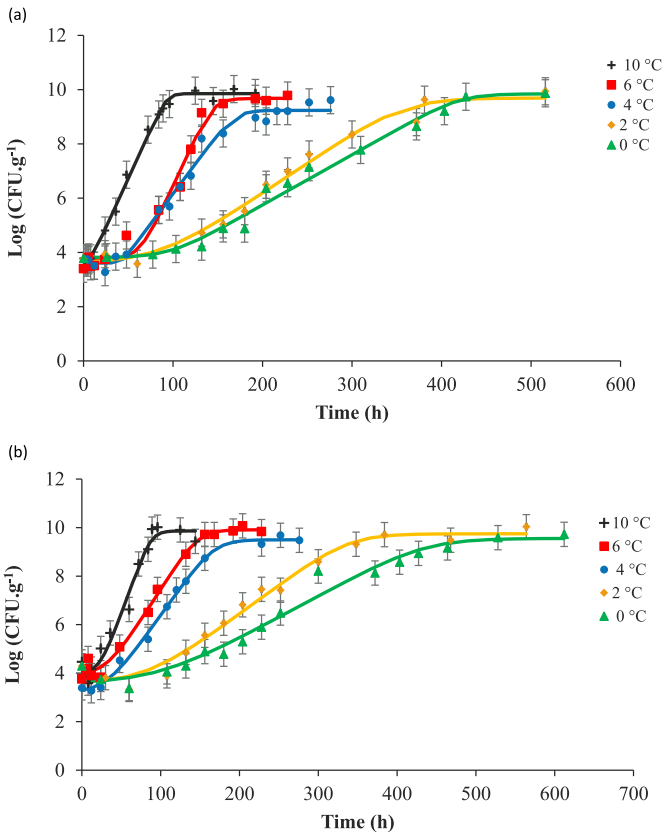

Fig. 1. Growth curves (mean ± se) of *Pseudomonas* spp. (a) and psychrotrophic bacteria (b) in chilled Pacu fillets at isothermal temperatures (0 °C, 2 °C, 4 °C, 6 °C, and + 10 °C), fitted (solid lines) with Baranyi and Roberts model.

The maximum population density (N_{max}) did not show a significant correlation with the storage temperature. This parameter is a time-dependent linear function for each obtained μ_{max}. While the lag time duration increased from 0.34 to 3.8 days at 10 °C and 0.6 to 4.4 days at 0C, respectively.

Temperature is the main factor affecting the kinetic parameters of bacterial growth (Koutsoumanis et al., 2006). Temperature affects the microbial enzymatic reactions, accelerating the metabolic rate. On the other hand, very low temperatures can freeze the plasma membrane and inhibit electron transport, the proton gradient, and enzymes (Zhang et al., 2011). Hence, as the storage temperature decreases, the microbial metabolism becomes slower. Likewise, several authors observed the dependence and significant influence of temperature on the μmax and t_{Lag} of *Pseudomonas* spp. in chilled fish or meat stored aerobically (Churchill et al., 2016; Zhang et al., 2011; Taoukis et al., 1999).

The major challenge of the fish industry is temperature control along the production chain (Anagnostopoulos et al., 2022; Zhuang et al., 2020). In Brazil, temperature monitoring in the fish industry is crucial for microbiological load control due to the tropical climate. Temperature fluctuations occur mainly in the interfaces of the operational process, that is, in refrigerated warehouses and markets. Such cold chain breaks are due to common logistical problems, such as the lack of good quality roads, the lack of proper temperature control, the lack of effectiveness of the authorities in inspecting these facilities, and commercial establishments that do not comply with legal temperature regulations. The ideal storage temperature to mitigate microbiological growth and ensure an adequate shelf-life for chilled fish in Brazil varies from 2 to 3 °C (Brasil, 2013). When the microbial population reaches about 10^7 CFU/g, the fish commonly presents signs of spoilage (ICSMS, IC on MS for F, 1986). In the present study, it has been observed that *Pseudomonas* spp. and psychotrophic bacteria reached a population of 10^7 CFU/g in

Table 2
Kinetic growth parameters of *Pseudomonas* spp. and psychotrophic bacteria in chilled pacu at isothermal temperatures (0–10 °C).

Temp. (°C)	*Pseudomonas* spp.				Psychrotrophic bacteria			
	μ_{max} (1/h)*	t_{Lag} (h)*	N_{max} (Log CFU/g)	R^2	μ_{max} (1/h)*	t_{Lag} (h)*	N_{max} (Log CFU/g)	R^2
0	0.016 ± 0.002[a]	93.19 ± 3.69[a]	9.97 ± 0.19[a]	0.97 ± 0.01	0.015 ± 0.000[a]	106.24 ± 5.19[a]	9.57 ± 0.19[a]	0.96 ± 0.00
2	0.025 ± 0.000[a]	76.33 ± 1.01[b]	9.88 ± 0.01[a]	0.98 ± 0.01	0.024 ± 0.006[ab]	87.56 ± 0.58[b]	9.88 ± 0.08[a]	0.98 ± 0.01
4	0.041 ± 0.004[b]	35.13 ± 4.67[c]	9.24 ± 0.04[a]	0.98 ± 0.01	0.035 ± 0.005[bc]	25.02 ± 3.99[c]	9.63 ± 0.19[a]	0.98 ± 0.12
6	0.053 ± 0.005[b]	19.66 ± 0.56[d]	9.68 ± 0.36[a]	0.99 ± 0.00	0.050 ± 0.003[c]	22.59 ± 2.14[c]	9.91 ± 0.06[a]	0.99 ± 0.00
10	0.075 ± 0.003[c]	8.21 ± 1.51[e]	9.87 ± 0.02[a]	0.99 ± 0.01	0.072 ± 0.002[d]	14.47 ± 4.56[c]	9.84 ± 0.04[a]	0.98 ± 0.01

μ_{max}: maximum specific growth rate, t_{Lag}: lag phase duration, N_{max}: maximum cell concentration.
* Different letters within the same column indicated significant ($P < 0.05$) differences among different storage temperatures revealed by Tuckey's test.

Pacu fillets stored at 2 °C with 7.5 and 10.6 days, respectively. Therefore, if the storage conditions can be adequately kept at these temperatures during commercialization, the fish stored at 2 °C should be consumed prior to 7 days. Nonetheless, it is known that temperature variations are common throughout the production and commercialization chain. Therefore, a secondary model was used to estimate the impact of temperature variation on the growth kinetic parameters of *Pseudomonas* spp. and psychotrophic bacteria.

Ratkowsky equation was used to describe the influence of temperature on the growth rate (μ_{max}) of *Pseudomonas* spp. and psychrotrophic bacteria. The models showed a strong linear correlation for the growth rate of *Pseudomonas* spp. and psychotrophic bacteria, with $R^2 > 0.97$. The estimated parameters are presented in Table 3 and minimum temperatures (T_{min}), −10.1 °C and −9.9 °C were estimated for *Pseudomonas* spp. and psychotrophic bacteria growth, respectively. Once the secondary models were developed, the study's next step prior to model use comprised the external validation. An external validation, varying the temperature profile, was performed to evaluate the performance of the models in predicting the handling, storage, and distribution of actual conditions. The modeling of *Pseudomonas* spp. and psychotrophic bacteria growth under non-isothermal conditions is presented in Fig. 2. The predicted results were compared with the observed growth rates (μ_{max}). Bias and accuracy factors were calculated, $B_f = 1.24$ and $A_f = 1.49$ for *Pseudomonas* spp., and $B_f = 1.45$ and $A_f = 1.49$ for psychotrophic bacteria. The bias factors >1 show that developed models result in fail-safe predictions, i.e., the models predicted the growth of *Pseudomonas* spp. and psychotrophic bacteria in refrigerated Pacu fillets before it occurs. For studies dealing with shelf-life estimation, the bias factor is expected to be >1 for the model to estimate the shelf-life before an organoleptic alteration occurs in the product (Zurera-Cosano et al., 2006). The present study demonstrated that the growth model for *Pseudomonas* spp. and psychotrophic bacteria can be used to make decisions regarding the shelf-life and quality control of refrigerated Pacu fillets under isothermal and dynamic conditions from 0 to 10 °C.

3.2. GC–MS analyzes

Several Volatile Organic Compounds (VOCs) are produced during storage and can be used as indicators of freshness or deterioration of the product. The main volatile compounds in fish are alcohols and aldehydes, and to a lesser extent ketones, aromatic compounds, and esters, among others (Selli and Cayhan, 2009). In the present study, 4 VOCs were selected for monitoring and quantification by gas chromatography: 3 alcohols (1-octen-2-ol, 1-hexanol, and 2-ethyl-1-hexanol) and 1 aldehyde (nonanal). These VOCs were chosen for monitoring since they exhibited an appropriate analytical signal. This signal presented a chromatographic peak with a relative area of at least 1 % of the highest peak area in the chromatogram. The obtained results (peak area) are presented in Table 4.

Table 3
Secondary model parameters of the effect of temperature (0–10 °C) on the *Pseudomonas* spp. and psychotrophic bacteria growth rates.

Microorganism	Square root models	R^2	R^2 adjusted
Pseudomonas spp.	$\sqrt{\mu_{máx}} = 0.016 \pm 0.0014\ (T+10.13 \pm 1.23)$	0.981	0.981
Psychotrophic bacteria	$\sqrt{\mu_{máx}} = 0.017 \pm 0.00084\ (T+9.91 \pm 0.68)$	0.974	0.974

μ_{max}: maximum specific growth rate (1/h), t_{Lag}: lag time duration in days, T (ºC) is temperature. Estimated parameters ± standard error.

Analyzing the influence of storage time, each one of the VOCs studied presented the same behavior over storage at the different temperatures analyzed (0, 4, and 10 °C). The nonanal concentration increased during chilled storage, showing a good correlation with the time. However, this increase was not significant ($P > 0.05$). The 1-octen-2-ol and 2-ethyl-1-hexanol compounds showed a reduction in the volatile fraction. This reduction was only significant for octenol at 0 °C and 2-ethyl-1-hexanol at 0 and 10 °C ($P < 0.05$). 1-Hexanol was the only volatile compound that did not present a standard behavior during chilled storage.

A reduction in the concentration of the alcohols (1-hexanol, octenol, and 2-ethyl-1-hexanol) was observed with the increase in temperature. This reduction was only significant for 2-ethyl-1-hexanol and octenol at intermediate points and 1-hexanol at the storage endpoint ($P < 0.05$).

The 1-octen-2-ol, 1-hexanol, 2-ethyl-1-hexanol, and nonanal compounds are derived from the oxidation of unsaturated fatty acids, naturally present in fish tissues (Fakruddin et al., 2013). Some studies reported that these VOCs may also be associated with the activity of *Pseudomonas* spp., *Carnobacterium*, and other fish spoilage, providing an additional contribution to the production of VOCs (Ercolini et al., 2011; Ercolini et al., 2010; Ferrocino et al., 2013; Odeyemi et al., 2018a, b). The behavior found for 1-hexanol and nonanal in the present study agrees with other studies conducted during fish storage (Li et al., 2018; Odeyemi et al., 2018a, b; Polo et al., 2014; Tuckey et al., 2013). In this study, a reduction in the concentration of 1-octen-2-ol and 2-ethyl-1hexanol was observed through the time of storage. According to Moreira et al. (2013), 1-octen-2-ol showed a significant concentration reduction after 2 weeks of storage. Moreover, Padda et al. (2001) reported that alcohols may be oxidized to ketones or esterified with carboxylic acids to ester during storage (Peterson and Chang, 1982), which justifies the reduction of these compounds.

Regarding the influence of temperature on VOC production, a reduction in the concentration of the alcohols (octenol, 2-ethyl-1-hexanol, and 1-hexanol) with the increase in temperature was observed. Selli and Cayhan (2009) reported that the production of volatile alcohols occurs by the action of the lipoxygenase enzyme in n − 3 and n − 6 polyunsaturated fatty acids. It is known that lipoxygenase activity is directly influenced by storage temperature. Hsieh et al. (1988) showed that at 0 °C, the lipoxygenase activity in rainbow trout gills corresponds to 60 %, thus a high enzymatic activity, while at temperatures between 10 °C, the activity is 90 %. Therefore, the increase in temperature contributes to a higher activity of the enzymes and, consequently, a higher production of VOCs. However, the reduction in the concentration of VOCs may be associated with the oxidation of alcohols to ketones, which occurs near the end of the storage period.

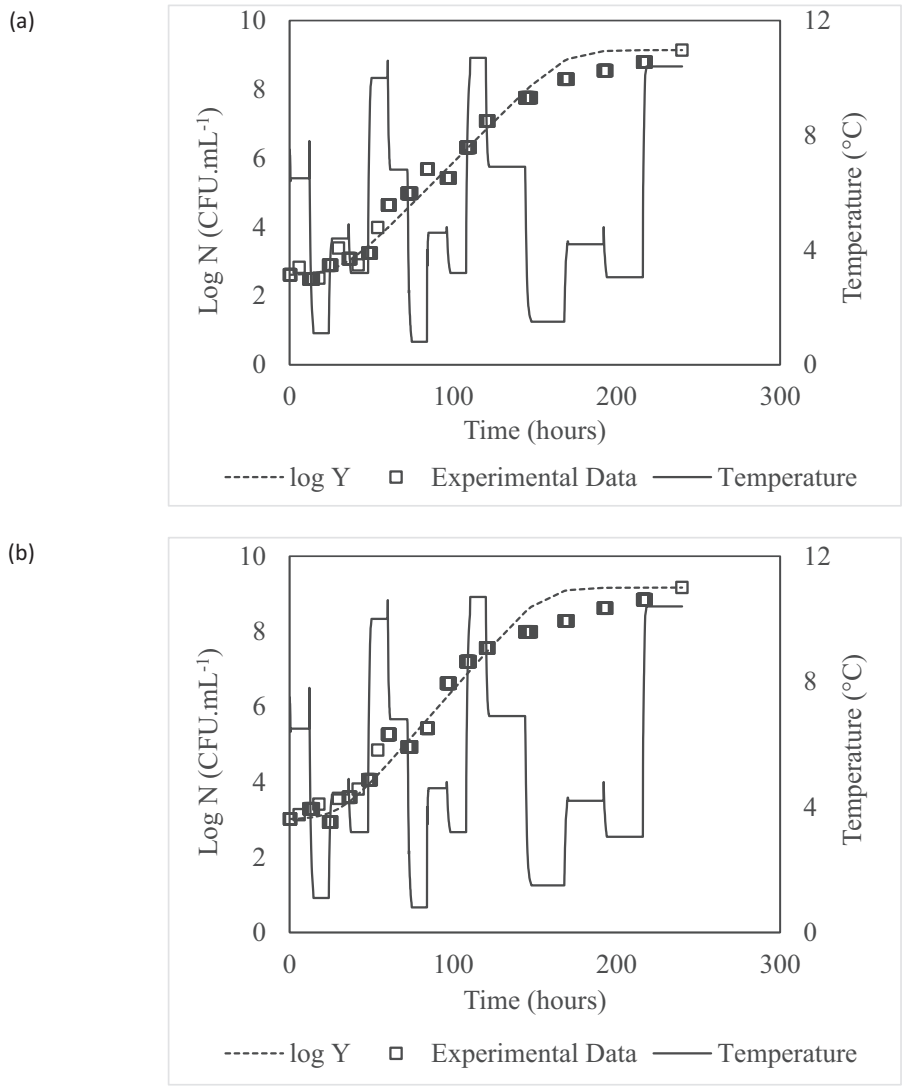

Fig. 2. The observed growth of *Pseudomonas* spp. (a), and psychrotrophic bacteria (b) on chilled Pacu fillets under non-isothermal storage conditions: (i) twice at periodically changing temperature (12 h at each temperature: 6 °C, 0 °C, 4 °C, 2 °C and 10 °C), totalizing120 hours, followed by (ii) 24 h at each temperature:6 °C, 0 °C, 4 °C, 2 °C and 10 °C, until the end of the shelf life.

3.3. ^{1}H NMR analyzes

The storage temperatures (2 °C and 8 °C) did not significantly impact the ^{1}H NMR profiles of the Pacu fillets (data not shown), which impeded the achievement of relevant component variability (crowded scores). Therefore, only the results obtained for the fish stored at the most drastic conditions (0 °C and 10 °C) were presented. Fig. 3 illustrates the metabolites assignment with a comparison among representatives ^{1}H NMR spectra of fish fillets stored at two different temperatures (0 °C and 10 °C) and sampled during two seasons (February/December and June). Different metabolites were assigned, such as amino acids, organic acids, and other compounds.

The primary identified compounds in Pacu fillets were lactic acid (doublet at δ 1.34 and quadruplet at δ 4.13), creatine and phosphocreatine (singlet at δ 3.05 and δ 3.95), and taurine (doublet of doublets at δ 3.30 and δ 3.44). In the aromatic region (δ 6.5–9.0), the main compounds detected were ATP/ADP (singlet at δ 8.57) and their decomposition products inosine (singlet at δ 8.48) and hypoxanthine (singlet at δ 8.23 and δ 8.24).

Pacu fillets stored at 0 °C presented higher amounts of ATP, lactic acid, creatine, and/or phosphocreatine, and lower amounts of inosine and hypoxanthine than the samples stored at 10 °C. Soon after fish slaughter, phosphocreatine is used as an energy source to generate ATP from ADP, with a consequent increase in creatine (Shah et al., 2010). In parallel, glucose is used to generate energy. The compound is cleaved anaerobically, resulting in the accumulation of lactic acid during storage. The reduction in lactic acid activity observed in the present study can be attributed to the microbiological activity of lactic acid bacteria and *Enterobacteriaceae*, which are reported to consume or produce lactate during post-mortem (Drosinos and Nychas, 1997). Subsequently, in the post-mortem, ATP will be used for muscle contractions and dephosphorylated in ADP and AMP through endogenous enzymes. The AMP will be decomposed, with chain formation of inosine and hypoxanthine, originating mainly from bacterial spoilage (Wyss and Kaddurah-Daouk, 2000). Therefore, the results of the present study indicate that Pacu fillets presented a greater degree of spoilage at the temperature of 10 °C than at 0 °C due to the increase of the activity of endogenous enzymes and microbial activity.

In addition, the samples stored at 10 °C showed higher amounts of valine, leucine, isoleucine, phenylalanine, alanine, acetic acid, taurine, and lower amounts of histidine when compared to fillet fish stored at 0 °C confirming that Pacu fillets present high spoilage degree at the highest temperature. Amino acids such as valine, leucine, isoleucine, phenylalanine, tyrosine, and alanine highly increase during the storage

Table 4
Volatile compounds in chilled pacu stored at 0, 4 and 10 °C.

Compounds	Sampling point	Temperature (°C)		
		0	4	10
1-Hexanol	Intermediate	21.10 ± 9.59^{aA}	49.51 ± 4.28^{aA}	1.40 ± 0.48^{aA}
	Final	343.34 ± 18.48^{aA}	18.51 ± 4.98^{aB}	2.09 ± 0.27^{aB}
Nonanal	Intermediate	8.14 ± 2.97^{aA}	4.75 ± 2.01^{aA}	2.91 ± 1.33^{aA}
	Final	14.44 ± 7.31^{aA}	6.00 ± 11.20^{aA}	5.84 ± 5.42^{aA}
Octenol	Intermediate	6.04 ± 2.35^{aA}	2.89 ± 1.21^{aB}	1.8 ± 1.17^{aB}
	Final	4.62 ± 1.86^{aA}	0.75 ± 0.24^{aA}	0.79 ± 0.64^{aA}
2Ethyl-1-hexanol	Intermediate	132.00 ± 25.10^{aA}	41.10 ± 1.80^{aB}	23.80 ± 5.08^{aB}
	Final	44.60 ± 34.90^{bA}	15.30 ± 4.65^{aA}	10.70 ± 1.36^{bA}

The values represent the means of the 2 fish batches, with two replicate packages and are reported as arbitrary units (10^3).
^{a,b}Different letters indicate significantly different between storage times ($P < 0.05$).
^{A,B}Different letters indicate significantly different among storage temperatures ($P < 0.05$).

due to protein hydrolysis, either by the action of proteolytic enzymes present in the food matrix or originated from the microbiological metabolism of the *Pseudomonas*, *Bacillus*, *Aeromonas*, and *Vibrio* spp. (Liao et al., 2018; Rao et al., 1998). Moreover, the histidine amino acid comes from decarboxylated amino acid histamine as a result of the activity of spoilage bacteria, such as *Morganella morganii*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Pseudomonas putrefaciens*, and *Clostridium perfringens* (Chen et al., 1987; Marrow et al., 1991; Middlebrooks et al., 1988). Additionally, acetic acid is metabolized in the fish matrix, exclusively associated with bacterial activity. Acetic acid production is associated with *B. thermosphacta*, lactic acid bacteria, *S. putrefaciens*, and *P. phosphoreum* growth (Gram et al., 2002; Macé et al., 2013; Nychas et al., 2008).

The taurine signal was also observed in Fig. 4. Taurine is a non-protein amino acid widely found in animal-origin products; its concentration differs widely between protein sources. This amino acid is not involved in autolysis reactions. However, it can be used by strains of *Pseudomonas* spp. as an energy source (Shimamoto and Berk, 1979). Additionally, some unknown metabolic activities contribute to its slight growth trend, according to the results in the present study at 10 °C (Tan et al., 2018).

Due to the complexity of the ^{1}H NMR data and the inherent similarity among the fish composition, the PCA method was performed to investigate the variability of the composition of Pacu fillets stored using different temperatures and different batches (December/February and June). Fig. 4 presents the PCA results for the evaluation of the storage temperatures on the fish fillets. The results showed essential variations based on the first two principal components (PCs) with 86.4 % of the total variance. Clear tendencies were observed in the scores graph (Fig. 4a) with the Pacu fillets stored at 0 °C in positive scores of PC1 and those stored at 10 °C in negative values of the same axis. The loadings graph (Fig. 4b) indicates that the fish stored at 0 °C presented the highest amounts of lactic acid, creatine phosphocreatine, and histidine. The storage temperature at 10 °C influenced the valine, leucine, isoleucine, alanine, acetic acid, taurine, and choline increase.

Additionally, an opposite behavior was detected between ATP and the compounds inosine, hypoxanthine, and phenylalanine in the aromatic region (δ 6.0–9.0). This finding can be explained by the fact that after fish death a series of enzymatic reactions lead to decomposition of ATP to inosine and hypoxanthine (Zhuang et al., 2021), which were favored by the temperature increase. Therefore, the Pacu fillets stored at 0 °C presented higher amounts of ATP and lower amounts of inosine, hypoxanthine, tyrosine, and phenylalanine than those stored at 10 °C.

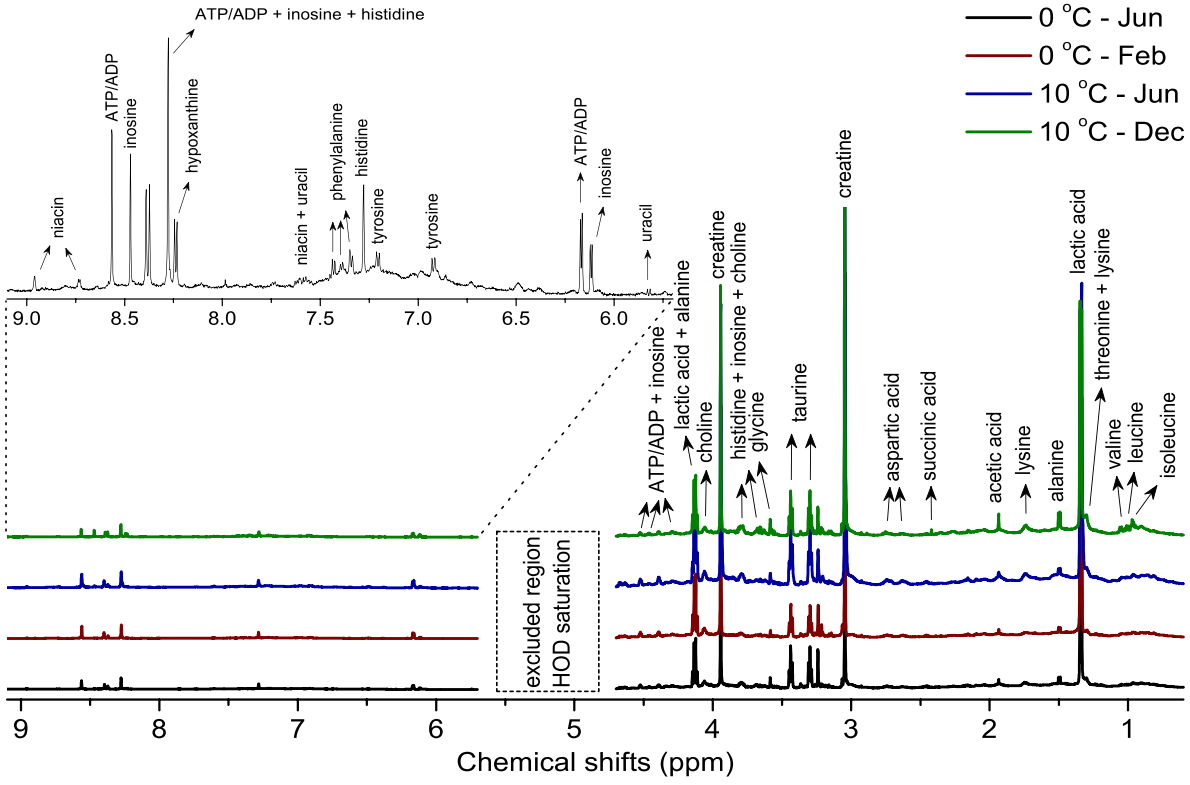

Fig. 3. Comparison of ^{1}H NMR spectra with identified compounds in chilled Pacu fillets stored at 0 °C and 10 °C and sampled from two different batches (December/February and June).

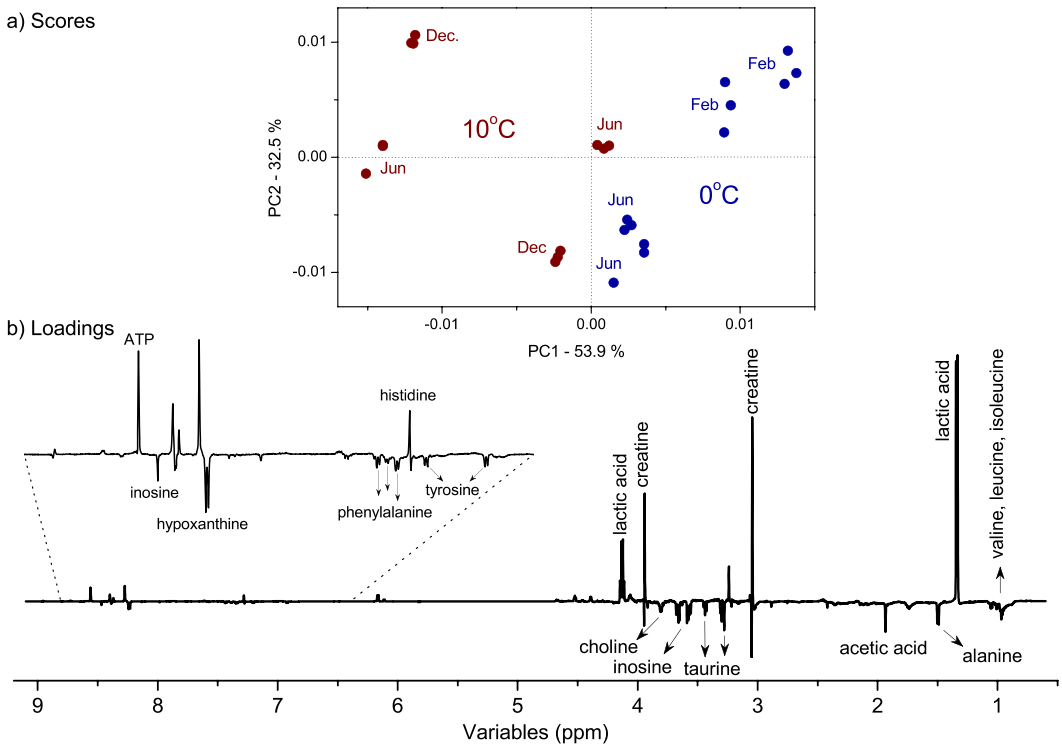

Fig. 4. PC1 × PC2 scores coordinate system (upside – a) and loadings (bottom – b) of the chilled Pacu fillets stored at 0 °C (blue color) and 10 °C (red color). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The compounds that presented variations in chemometric analysis and did not exhibit overlapping resonances were quantified, and the results are shown in Fig. 4. Based on the ANOVA single factor, the same tendencies observed in PCA results were detected in quantitative results (corroborated analyses). This finding demonstrated the advantage of multivariate analysis compared to univariate analysis (as quantification) for evaluating the organic variability in foodstuff matrices. In addition, chemometric analysis was applied to quickly obtain information by high-quality evaluation of the organic changes, improving the comprehension of the effect of different storage conditions. This approach is helpful since the compounds and their degradation products are only sometimes known, and the acquisition of certified standards is sometimes complex.

4. Conclusion

The present study demonstrated that the predictive growth model developed for *Pseudomonas* spp. and psychrotrophic bacteria can be used as a tool for decision-making on the quality of refrigerated Pacu fillets under isothermal and dynamic conditions from 0 to 10 °C. The ^{1}H NMR analysis indicated that Pacu fillets presented higher spoilage degrees at 10 °C than at 0 °C. Specific compounds indicated an increased activity of endogenous enzymes and microbiological activity. There was also an increase in non-volatile metabolites during storage, highlighting the impact of temperature and time on the spoilage of Pacu fillets during commercialization.

CRediT authorship contribution statement

Rafaela C. Baptista: Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Rodrigo B.A. Oliveira:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Antonio A. Câmara:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Émilie Lang:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Juliana L.P. dos Santos:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Matheus Pavani:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Tatiane M. Guerreiro:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Rodrigo R. Catharino:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Elenilson G.A. Filho:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Sueli Rodrigues:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Edy S. de Brito:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Verônica O. Alvarenga:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Gerson B. Bicca:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Anderson S. Sant'Ana:** Writing – review & editing, Writing – original draft,

Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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References

Alves Filho, E.G., Almeida, F.D.L., Cavalcante, R.S., Brito, E.S. De, Cullen, P.J., Frias, J. M., Bourke, P., Fernandes, F.A.N., Rodrigues, S., 2016. H NMR spectroscopy and chemometrics evaluation of non-thermal processing of orange juice. Food Chem. 204, 102–107. https://doi.org/10.1016/j.foodchem.2016.02.121.

Alves Filho, E.G., Silva, L.M.A., Teo, E.M., Larsen, F.H., Brito, E.S. De, 2017. Genotype evaluation of cowpea seeds (*Vigna unguiculata*) using 1 H q NMR combined with exploratory tools and solid-state NMR. Food Res. Int. 91, 140–147. https://doi.org/10.1016/j.foodres.2016.12.007.

Anagnostopoulos, D.A., Parlapani, F.F., Boziaris, I.S., 2022. The evolution of knowledge on seafood spoilage microbiota from the 20th to the 21st century: have we finished or just begun? Trends Food Sci. Technol. 120, 236–247. https://doi.org/10.1016/j.tifs.2022.01.004.

Baptista, R.C., Horita, C.N., Sant'Ana, A.S., 2020. Natural products with preservative properties for enhancing the microbiological safety and extending the shelf-life of seafood: a review. Food Res. Int. 127, 108762 https://doi.org/10.1016/j.foodres.2019.108762.

Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23, 277–294. https://doi.org/10.1016/0168-1605(94)90157-0.

Baranyi, J., Robinson, T.P., Kaloti, A., Mackey, B.M., 1995. Predicting growth of *Brochothrix thermosphacta* at changing temperature. Int. J. Food Microbiol. 27, 61–75. https://doi.org/10.1016/0168-1605(94)00154-X.

Brasil, 2013. Portaria CVS 5, de 09 de abril de 2013 DOE de 19/04/2013 - n°. In: 73 - Poder Executivo – Seção I. Vigilância Sanitária, Cent.

Cai, H., Chen, Y., Cui, X., Cai, S., Chen, Z., 2014. High-resolution 1 H NMR spectroscopy of fish muscle, eggs and small whole fish via Hadamard-encoded intermolecular multiple-quantum coherence. PLoS One 9, 1–7. https://doi.org/10.1371/journal.pone.0086422.

Calcagnotto, D., DeSalle, R., 2009. Population genetic structuring in PacuPacu (*Piaractus mesopotamicus*) across the Paraná-Paraguay basin: evidence from microsatellites. Neotrop. Ichthyol. 7, 607–616. https://doi.org/10.1590/S1679-62252009000400008.

Chen, C.M., Marshall, M.R., Koburger, J.A., Otwell, W.S., Wei, C.I., 1987. Determination of minimal temperatures for histamine production by five bacteria. In: Otwell, W.T. (Ed.), First Joint Conf. With Atlantic Fisheries Technol. Soc, pp. 365–376.

Churchill, O.J., Fernandez-Piquer, J., Powell, S.M., Tamplin, M.L., 2016. Microbial and sensorial models for head-on and gutted (HOG) Atlantic Salmon (*Salmo salar*) stored from 0 to 15 °C. Food Microbiol. 57, 144–150. https://doi.org/10.1016/j.fm.2016.02.006.

Dalgaard, P., Madsen, H.L., Samieian, N., Emborg, J., 2006. Biogenic amine formation and microbial spoilage in chilled garfish (*Belone belone belone*) – effect of modified atmosphere packaging and previously frozen storage. J. Appl. Microbiol. 101, 80–95. https://doi.org/10.1111/j.1365-2672.2006.02905.x.

Doulgeraki, A.I., Paramithiotis, S., Maria, D., Nychas, G.E., 2010. Lactic acid bacteria population dynamics during minced beef storage under aerobic or modified atmosphere packaging conditions. Food Microbiol. 27, 1028–1034. https://doi.org/10.1016/j.fm.2010.07.004.

Doyle, M.P., Beuchat, L.R., Montville, T.J., Press, A., 2003. Food Microbiology: Frontiers, in Trends in Food Science & Technology, pp. 390–391.

Drosinos, E.H., Nychas, G.J.E., 1997. Production of acetate and lactate in relation to glucose content during modified atmosphere storage of gilthead seabream (*Sparus aurata*) at 0 ± 1°C. Food Res. Int. 30, 711–717. https://doi.org/10.1016/S0963-9969(98)00036-2.

Ercolini, D., Casaburi, A., Nasi, A., Ferrocino, I., Ferranti, P., Mauriello, G., Villani, F., 2010. Different molecular types of *Pseudomonas fragi* have the same overall behaviour as meat spoilers. Int. J. Food Microbiol. 142, 120–131. https://doi.org/10.1016/j.ijfoodmicro.2010.06.012.

Ercolini, D., Ferrocino, I., Nasi, A., Ndagijimana, M., Vernocchi, P., Storia, A. La, Laghi, L., Mauriello, G., Guerzoni, M.E., Villani, F., 2011. Monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions monitoring of microbial metabolites and bacterial diversity in beef stored under different packaging conditions. Appl. Environ. Microbiol. 77, 7372–7381. https://doi.org/10.1128/AEM.05521-11.

Fakruddin, M.D., Sultana, M., Ahmed, M.M., Chowdhury, A., Choudhury, N., 2013. Multiplex PCR (polymerase chain reaction) assay for detection of *E. coli* O157:H7, *Salmonella* sp., *Vibrio cholerae* and *Vibrio parahaemolyticus* in spiked shrimps (*Penaeus monodon*). Pak. J. Biol. Sci. 16, 267–274. https://scialert.net/abstract/?doi=pjbs.2013.267.274.

Ferrocino, I., La Storia, A., Torrieri, E., Musso, S.S., Mauriello, G., Villani, F., Ercolini, D., 2013. Antimicrobial packaging to retard the growth of spoilage bacteria and to reduce the release of volatile metabolites in meat stored under vacuum at 1°C. J. Food Prot. 76, 52–58. https://doi.org/10.4315/0362-028X.JFP-12-257.

Gill, C., Mcginnis, J.C., Rahn, K., Houde, A., 1997. Control of product temperatures during the storage and transport of bulk containers of manufacturing beef. Food Res. Int. 29, 647–651. https://doi.org/10.1016/S0963-9969(96)00071-3.

Gram, L., Dalgaard, P., 2002. Fish spoilage bacteria - problems and solutions. Curr. Opin. Biotechnol. 13, 262–266. https://doi.org/10.1016/S0958-1669(02)00309-9.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., Givskov, M., 2002. Food spoilage–interactions between food spoilage bacteria. Int. J. Food Microbiol. 78, 79–97. https://doi.org/10.1016/S0168-1605(02)00233-7.

Hotelling, H., 1933. Analysis of a complex of statistical variables into principal components. J. Educ. Psychol. 24, 417–441. https://doi.org/10.1037/h0071325.

Hsieh, R.J., German, J.B., Kinsella, J.E., 1988. Lipoxygenase in fish tissue: some properties of the 12-lipoxygenan from trout gill. J. Agric. Food Chem. 36, 680–685. https://doi.org/10.1021/jf00082a003.

ICSMS, IC on MS for F., 1986. Sampling plans for fish and shellfish. In: Microorganisms in foods, in International Union of Biological Societies (Ed.), Sampling for Microbiological Analysis: Principles and Specific Applications. Blackwell Scientific Publications, Oxford, UK, pp. 181–196.

James, S.J., James, C., Evans, J.A., 2006. Modelling of food transportation systems e a review´lisation des syste`mes de transport utilise's pour Revue de la mode´es alimentaires les denre. Int. J. Refrig. 29, 947–957. https://doi.org/10.1016/j.ijrefrig.2006.03.017.

Koutsoumanis, K., Nychas, G.J.E., 1999. Chemical and sensory changes associated with microbial flora of Mediterranean boque (Boops boops) stored aerobically at 0, 3, 7, and 10°C. Appl. Environ. Microbiol. 65, 698–706. https://doi.org/10.1128/AEM.65.2.698-706.1999.

Koutsoumanis, K., Stamatiou, A., Skandamis, P., Nychas, G.E., 2006. Development of a microbial model for the combined effect of temperature and pH on spoilage of ground meat and validation of the model under dynamic temperature conditions. Appl. Environ. Microbiol. 72, 124–134. https://doi.org/10.1128/AEM.72.1.124.

Li, Q., Zhang, L., Luo, Y., 2018. Changes in microbial communities and quality attributes of white muscle and dark muscle from common carp (*Cyprinus carpio*) during chilled and freeze-chilled storage. Food Microbiol. 73, 237–244. https://doi.org/10.1016/j.fm.2018.01.011.

Liao, Q., Suzuki, T., Shirataki, Y., Kuramoto, M., Kondo, N., 2018. Freshness-related fluorescent compound changes in Japanese dace fish (*Tribolodon hakonensis*) eye fluid during storage. Eng. Agric. Environ. Food. 11, 95–100. https://doi.org/10.1016/j.eaef.2018.01.001.

Lin, C.Y., Wu, H., Tjeerdema, R.S., Viant, M.R., 2017. Evaluation of metabolite extraction strategies from tissue samples using NMR metabolomics. Metabolomics 3, 55–67. https://doi.org/10.1007/s11306-006-0043-1.

Macé, S., Joffraud, J., Cardinal, M., Malcheva, M., Cornet, J., Lalanne, V., Chevalier, F., Sérot, T., Pilet, M., Dousset, X., 2013. Evaluation of the spoilage potential of bacteria isolated from spoiled raw salmon (*Salmo salar*) fillets stored under modified atmosphere packaging. Int. J. Food Microbiol. 160, 227–238. https://doi.org/10.1016/j.ijfoodmicro.2012.10.013.

Marrow, J., Margolies, G., Rowland, J., Roberts, L., 1991. Evidence that histamine is the causative toxin of scombroid-fish poisoning. N. Engl. J. Med. 11, 716–720. https://doi.org/10.1056/NEJM199103143241102.

Middlebrooks, B.L., Toom, P.M., Douglas, W.L., Harrison, R.E., McDowell, S., 1988. Effects of storage time and temperature on the microflora and amine-development in Spanish mackerel (*Scomberomorus maculatus*). J. Food Sci. Food Sci. 53, 1024–1029. https://doi.org/10.1111/j.1365-2621.1988.tb13522.x.

Mikš-Krajnik, M., Yoon, Y.J., Ukuku, D.O., Yuk, H.G., 2016. Volatile chemical spoilage indexes of raw Atlantic salmon (*Salmo salar)* stored under aerobic condition in relation to microbiological and sensory shelf lives. Food Microbiol. 53, 182–191. https://doi.org/10.1016/j.fm.2015.10.001.

Moreira, N., Valente, L.M.P., Castro-cunha, M., Cunha, L.M., Pinho, P.G. De, 2013. Effect of storage time and heat processing on the volatile profile of Senegalese sole (*Solea senegalensis* Kaup, 1858) muscle. Food Chem. 138, 2365–2373. https://doi.org/10.1016/j.foodchem.2012.11.135.

Nychas, G.E., Skandamis, P.N., Tassou, C.C., Konstantinos, K.P., 2008. Meat spoilage during distribution. Meat Sci. 78, 77–89. https://doi.org/10.1016/j.meatsci.2007.06.020.

Odeyemi, O.A., Burke, C.M., Bolch, C.C.J., Stanley, R., 2018a. Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. Int. J. Food Microbiol. 280, 87–99. https://doi.org/10.1016/j.ijfoodmicro.2017.12.029.

Odeyemi, O.A., Burke, C.M., Bolch, C.J.S., Stanley, R., 2018b. Evaluation of spoilage potential and volatile metabolites production by *Shewanella baltica* isolated from modified atmosphere packaged live mussels. Food Res. Int. 103, 415–425. https://doi.org/10.1016/j.foodres.2017.10.068.

Padda, R.S., Pandey, K.K., Kaul, S., Nair, V.D., Jain, R.K., Basu, S.K., Chakrabarti, T., 2001. A novel gene encoding a 54 kDa polypeptide is essential for butane utilization by *Pseudomonas* sp. IMT37. Microbiology 147, 2479–2491. https://doi.org/10.1099/00221287-147-9-2479.

Parlapani, F.F., 2021. Microbial diversity of seafood. Curr. Opin. Food Sci. 37, 45–51. https://doi.org/10.1016/j.cofs.2020.09.005.

Parlapani, F.F., Kormas, K.A., Boziaris, I.S., 2014. Microbiological changes, shelf life, and identification of initial and spoilage microbiota of sea bream fillets stored under various conditions using 16S rRNA gene analysis. J. Sci. Food Agric. 95, 2386–2394. https://doi.org/10.1002/jsfa.6957.

Peterson, R.J., Chang, S.S., 1982. Identification of volatile flavor compounds of fresh, frozen beef stew and comparison of these with those of canned beef stew. J. Food Sci. 47, 1444–1448. https://doi.org/10.1111/j.1365-2621.1982.tb04957.x.

Pino Hernández, E.J.G., Carvalho, R.N. de, Joele, M.R.S.P., Araújo, C. da S., Lourenço, L. de F.H., 2017. Effects of modified atmosphere packing over the shelf life of sous vide from captive pirarucu (*Arapaima gigas*). Innov. Food Sci. Emerg. Technol., 39, 94–100. doi:https://doi.org/10.1016/j.ifset.2016.11.009.

Polo, D., Feal, X., Varela, M.F., Monteagudo, A., Romalde, J.L., 2014. Depuration kinetics of murine norovirus in shellfish. Food Res. Int. 64, 182–187. https://doi.org/10.1016/j.foodres.2014.06.027.

Possas, A., Valero, A., Pérez-Rodríguez, F., 2022. New software solutions for microbiological food safety assessment and management. Curr. Opin. Food Sci. 44, 100814 https://doi.org/10.1016/j.cofs.2022.100814.

Raab, V., Bruckner, S., Beierle, E., Kampmann, Y., Petersen, B., Kreyenschmidt, J., 2008. Generic model for the prediction of remaining shelf life in support of cold chain management in pork and poultry supply chains. J. Chain Netw. Sci. 8, 59–74. https://doi.org/10.3920/JCNS2008.x089.

Rao, M.B., Tanksale, A.M., Ghatge, M.S., Deshpande, V.V., 1998. Molecular and biotechnological aspects of microbial proteases. Microbiol. Mol. Biol. Rev. 62, 597–635. https://doi.org/10.1128/mmbr.62.3.597-635.1998.

Ratkowsky, D.A., Olley, J., Mcmeekin, T.A., Ball, A., 1982. Relationship between temperature and growth rate of bacterial cultures. J. Bacteriol. 149, 1–5. https://doi.org/10.1128/jb.149.1.1-5.1982.

Rediers, H., Claes, M., Peeters, L., Willems, K.A., 2009. Postharvest biology and technology evaluation of the cold chain of fresh-cut endive from farmer to plate. Postharvest Biol. Technol. 51, 257–262. https://doi.org/10.1016/j.postharvbio.2008.07.017.

Rodrigues, B.L., da Costa, M.P., da Silva Frasão, B., da Silva, F.A., Mársico, E.T., da Silveira Alvares, T., Conte-Junior, C.A., 2017. Instrumental texture parameters as freshness indicators in five farmed Brazilian freshwater fish species. Food Anal. Methods 10 (11), 3589–3599. https://doi.org/10.1007/s12161-017-0926-y.

Ross, T., 1996. Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol. 81, 501–508. https://doi.org/10.1111/j.1365-2672.1996.tb03539.x.

Scilab Enterprises, 2012. Scilab: Free and Open Source Software for Numerical Computation (Windows, Version 6.1.0). Software.

Selli, S., Cayhan, G.G., 2009. Analysis of volatile compounds of wild gilthead sea bream (*Sparus aurata*) by simultaneous distillation extraction (SDE) and GC-MS. Microchem. J. 93, 232–235. https://doi.org/10.1016/j.microc.2009.07.010.

Shah, A.K.M.A., Ogasawara, M., Egi, M., Kurihara, H., Takahashi, K., 2010. Identification and sensory evaluation of flavor enhancers in Japanese traditional dried herring (*Clupea pallasii*) fillet. Food Chem. 122, 249–253. https://doi.org/10.1016/j.foodchem.2010.02.072.

Shimamoto, G., Berk, R.S., 1979. Catabolism of taurine in *Pseudomonas aeruginosa*. Biochim. Biophys. Acta 569, 287–292. https://doi.org/10.1016/0005-2744(79)90064-0.

Shumilina, E., Slizyte, R., Mozuraityte, R., Dykyy, A., Stein, T.A., Dikiy, A., 2016. Quality changes of salmon by-products during storage: assessment and quantification by NMR. Food Chem. 211, 803–811. https://doi.org/10.1016/j.foodchem.2016.05.088.

Sucupira, N.R., Filho, E.G.A., Silva, L.M.A., Brito, E.S. De, Wurlitzer, N.J., Sousa, P.H.M., 2017. NMR spectroscopy and chemometrics to evaluate different processing of coconut water. Food Chem. 216, 217–224. https://doi.org/10.1016/j.foodchem.2016.08.035.

Tan, C., Huang, Y., Feng, J., Li, Z., Cai, S., 2018. Chemical freshness assessment of intact fish via 2D 1 H J -resolved NMR spectroscopy combined with pattern recognition methods. Sensors Actuators B Chem. 255, 348–356. https://doi.org/10.1016/j.snb.2017.08.060.

Tanamati, A., Stevanato, F.B., Eliete, J., Visentainer, L., Matsushita, M., Souza, N.E. De, Visentainer, J.V., 2009. Fatty acid composition in wild and cultivated Pacu and pintado fish. Eur. J. Lipid Sci. Technol. 111, 183–187. https://doi.org/10.1002/ejlt.200800103.

Taoukis, P.S., Koutsoumanis, K., Nychas, G.J.E., 1999. Use of time–temperature integrators and predictive modeling for shelf life control of chilled fish under dynamic storage conditions. Int. J. Food Microbiol. 53, 21–31. https://doi.org/10.1016/S0168-1605(99)00142-7.

Tenenhaus-Aziza, F., Ellouze, M., 2015. Software for predictive microbiology and risk assessment: a description and comparison of tools presented at the ICPMF8 Software Fair. Food Microbiol. 45, 290–299. https://doi.org/10.1016/j.fm.2014.06.026.

Tsironi, T., Houhoula, D., Taoukis, P., 2020. Hurdle technology for fish preservation. Aquac. Fish. 5 (2), 65–71. https://doi.org/10.1016/j.aaf.2020.02.001.

Tuckey, N.P.L., Day, J.R., Miller, M.R., 2013. Determination of volatile compounds in New Zealand Greenshell TM mussels (*Perna canaliculus*) during chilled storage using solid phase microextraction gas-chromatography–mass spectrometry. Food Chem. 136, 218–223. https://doi.org/10.1016/j.foodchem.2012.07.118.

Urbinati, E.C., Gonçalves, F.D., 2005. Pacu (*Piaractus mesopotamicus*). In: Baldisserotto, B., Gomes, L.C. (Eds.), Espécies Nativas para Piscicultura no Brasil. Editora UFSM, Santa Maria, pp. 225–255.

Wagner, L., Trattner, S., Pickova, J., Gómez-requeni, P., Moazzami, A.A., 2014. H NMR-based metabolomics studies on the effect of sesamin in Atlantic salmon (*Salmo salar*). Food Chem. 147, 98–105. https://doi.org/10.1016/j.foodchem.2013.09.128.

Wei, Y., Liang, M., Mai, K., Zheng, K., Xu, H., 2017. 1H NMR-based metabolomics studies on the effect of size-fractionated fish protein hydrolysate, fish meal and plant protein in diet for juvenile turbot (*Scophthalmus maximus* L.). Aquac. Nutr. 23, 523–536. https://doi.org/10.1111/anu.12420.

Wyss, M., Kaddurah-Daouk, R., 2000. Creatine and creatinine metabolism. Physiol. Rev. 80, 1107–1213. https://doi.org/10.1152/physrev.2000.80.3.1107.

Xu, Y., Liu, Y., Zhang, C., Li, X., Yi, S., Li, J., 2015. Physicochemical responses and quality changes of turbot (*Psetta maxima*) during refrigerated storage. Int. J. Food Prop. 19, 196–209. https://doi.org/10.1080/10942912.2015.1022260.

Yu, D., Xu, Y., Jiang, Q., Yang, F., Xia, W., 2016. Freshness assessment of grass carp (Ctenopharyngodon idellus) fillets during storage at 4 °C by physicochemical, microbiological and sensorial evaluations. J. Food Saf. 37, 2. https://doi.org/10.1111/jfs.12305.

Zhang, Y., Mao, Y., Li, K., Dong, P., Liang, R., Luo, X., 2011. Models of *Pseudomonas* growth kinetics and shelf life in chilled longissimus dorsi muscles of beef. Asian-Australasian J. Anim. Sci. 24, 713–722. https://doi.org/10.5713/ajas.2011.10404.

Zhuang, S., Hong, H., Zhang, L., Luo, Y., 2020. Spoilage-related microbiota in fish and crustaceans during storage: research progress and future trends. Compr. Rev. Food Sci. Food Saf. 20 (1), 252–288. https://doi.org/10.1111/1541-4337.12659.

Zhuang, S., Liu, X., Li, Y., Zhang, L., Hong, H., Liu, J., Luo, Y., 2021. Biochemical changes and amino acid deamination & decarboxylation activities of spoilage microbiota in chill-stored grass carp (Ctenopharyngodon idella) fillets. Food Chem. 336, 127683 https://doi.org/10.1016/j.foodchem.2020.127683.

Zuanazzi , 2013. Determinação da composição centesimal de pacu (*Piaractus mesopotamicus*) cultivados em tanques-rede no Pantanal. SIMPÓSIO SOBRE RECURSOS NATURAIS E SOCIOECONÔMICOS DO PANTANAL, 6.; EVENTO DE INICIAÇÃO CIENTÍFICA DO PANTANAL, 1.

Zurera-Cosano, G., García-Gimeno, R.M., Rodríguez-Pérez, R., Hervás-Martínez, C., 2006. Performance of response surface model for prediction of *Leuconostoc mesenteroides* growth parameters under different experimental conditions. Food Control 17, 429–438. https://doi.org/10.1016/j.foodcont.2005.02.003.