

# Bleeding Influencing Color, Physical-Chemical Quality, and Texture Profile of Pirarucu (*Arapaima Gigas*) from Amazon, Brazil

Pamela Esteves Bassil

Faculdade de Veterinária, Universidade Federal Fluminense, Brazil

Mayara Xavier Martins

Faculdade de Veterinária, Universidade Federal Fluminense, Brazil

Paula Hernandes Sandes

Faculdade de Veterinária, Universidade Federal Fluminense, Brazil

Ana Paula Amaral de Alcântara Salim

Faculdade de Veterinária, Universidade Federal Fluminense, Brazil

Eliane Teixeira Mássico

Faculdade de Veterinária, Universidade Federal Fluminense, Brazil

Sérgio Borges Mano

Faculdade de Veterinária, Universidade Federal Fluminense, Brazil

Camila Valente Alva

Faculdade de Veterinária, Universidade Federal Fluminense, Brazil

Bruno Vianna Barradas

Faculdade de Veterinária, Universidade Federal Fluminense, Brazil

João Vitor Campos-Silva

Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas.

Faculty of Environmental Sciences and Natural Resource Management, Norwegian

University of Life Sciences, Ås, Norway

Brazil

Ana Paula Campos Barros

Instituto de Desenvolvimento Sustentável Mamirauá, Brazil

Antônio Adevaldo Dias da Costa

Memorial Chico Mendes, Brazil

Micheli da Silva Ferreira (Corresponding author)

Departamento de Tecnologia de Alimentos, Faculdade de Veterinária, Universidade Federal Fluminense, Brazil

Received: May 4, 2022    Accepted: June 7, 2022    Published: June 23, 2022

doi:10.5296/jfs.v11i1.19832    URL: <https://doi.org/10.5296/jfs.v11i1.19832>

## Abstract

Pirarucu (*Arapaima gigas*) fishing and meat contribute socio-economically to the Amazon population, with recovering stocks resulting from the pirarucu management in the Middle Juruá region. The ventral portion of arapaima (belly) is widely consumed due to the high lipid content, which can predispose lipid and protein oxidative reactions. Differences among fishing practices, including the bleeding, may influence the quality and acceptance of the meat. In this context, we evaluated the effect of bleeding on the physical-chemical quality, color, and texture of bellies of arapaima obtained from sustainable community-based management. The bellies were obtained from ten (n=10) pirarucu carcasses. Five (n = 5) animals were slaughtered with bleeding by the gills (BLE) and five (n = 5) animals without bleeding (NON-BLE). Pirarucu bellies were sliced and assigned randomly for 0, 3, 6, and 9 days at 4°C to analyze pH, water holding capacity, instrumental color, and texture profile. NON-BLE exhibited greater ( $P < 0.05$ ) redness and yellowness than BLE samples, whereas BLE exhibited greater ( $P < 0.05$ ) color stability than their correlative BLE samples. BLE bellies exhibited greater ( $P < 0.05$ ) hardness and chewiness than in NON-BLE counterparts.

During storage, both BLE and NON-BLE exhibited an increase ( $P < 0.05$ ) in pH. BLE bellies demonstrated a decrease ( $P < 0.05$ ) in yellowness and color stability, whereas an increase ( $P < 0.05$ ) in hardness and chewiness were observed in the same samples. These findings indicated that bleeding could improve the pirarucu meat quality.

**Keywords:** pirarucu, oxidative stability, texture profile, CIELAB

## 1. Introduction

Pirarucu (*Arapaima gigas*) is the largest scale freshwater fish globally, widely appreciated due to their meat quality, rusticity, and carcass yield, which consumption contributes to a positive social-economic impact on the Amazon population (Fogaça et al., 2011). In this context, the community-based management and conservation of fish stocks are crucial to ensure the socio-economic stability and wellbeing of wetlands dwellers. A remarkable example of the empowerment of artisanal fish is the arapaima management in the Middle Juruá region (Amazon, Brazil), where lake protection status contributed to enhancing the population of arapaima in protected areas (Campos-Silva & Peres, 2016).

The commercial cuts of arapaima include loin (dorsal portion), tail, and belly (ventral portion). The ventral portion is widely appreciated and consumed due to its high lipid content than the fillet. Although these characteristics enhance the palatability of belly meat, it also favors undesirable degradative changes such as lipid and protein oxidation (Wei et al. 2017).

Differences among fishing practices may contribute to oxidative changes decreasing carcass quality and acceptance of final products. However, the bleeding practice during the pirarucu fisheries does not occur in all management areas. The lack of bleeding would favor the presence of residual blood in carcasses decreasing the product shelf-life and favoring their susceptibility to oxidative reactions and meat discoloration (Addeen et al., 2017).

The influence of bleeding on surface color (Sterniša et al., 2018; Jiang et al., 2019; Roth et al., 2007; Erikson et al., 2010; Ramos et al., 2005) and texture (Jing et al., 2013) has been previously documented in fillets of common carp (*Cyprinus carpio*; Sterniša et al., 2018), yellowtail (*Seriola quinquediata*; Jiang et al., 2019), farmed turbot (*Scophthalmus maximus*; Roth et al., 2007), Atlantic salmon (*Salmo salar*; Erikson et al., 2010), bullfrog (*Rana catesbeiana*; Ramos et al., 2005) and tilapia (*Oreochromis niloticus*; Jing et al., 2013). However, the influence of bleeding on color and texture profile parameters of pirarucu bellies is yet to be investigated. Here, we investigate the color and texture profile parameters of pirarucu bellies from animals harvested with and without bleeding during refrigerated storage. Our results help to improve the community-based management of pirarucu towards a better fishing practice.

## 2. Method

### 2.1 Study Area and Arapaima Management Context

This work was part of a collective effort coordinated by the Coletivo of Pirarucu to understand the factors that influence the quality of pirarucu's meat, mainly the meat commercialized under coordination by Associação dos Produtores Rurais do Carauari

(ASPROC). This research, carried out in collaboration with the Universidade Federal Fluminense (UFF), resulted from an extensive survey. Some of the results are being reported here and had the contribution of several institutions.

In this context, this study was carried out at a sustainable-use reserve of the Juru á River. The region is a federally-managed Extractive Reserve - RESEX of Middle Juru á, legally occupied by riverside communities. In the Middle Juru á region, fishing accords were established between local communities and the ASPROC to guarantee food and economic security. Annually, occurs the pirarucu management by the resident community. The number of fish allowed to be captured is based on the number of pirarucus (adults and juveniles) registered at that lake in the previous year (Campos-Silva & Peres, 2016).

Due to the large geographic dimensions of this RESEX and the number of communities involved, many practices can influence the final quality of pirarucu's meat. Therefore, the goal of the present study was to evaluate the influence of bleeding on the quality of pirarucu bellies to understand the factors that influence the meat quality of pirarucu.

## 2.2 Experimental Design

The pirarucus were harvested at Middle Juru á Amazon, Brazil, processed and frozen in a fish industry under Brazilian Federal Inspection located in Manacapuru (AM, Brazil). The bellies samples were purchased and transported frozen to Universidade Federal Fluminense (Niteroi, Rio de Janeiro, Brazil). Thus, institutional animal care and use committee approval was not obtained.

Ten pirarucus (*Arapaima gigas*) were used in this experiment. The animals were harvested by local fishermen at the Middle Juru á river and processed under Brazilian Federal Inspection at a fish industry. Five ( $n = 5$ ) animals were harvested, followed by bleeding by the gills (BLE), and five ( $n = 5$ ) animals were harvested without bleeding (NON-BLE). Fish samples were sliced into eight 2.54-cm samples of ventral portion cut (bellies). The bellies were individually packaged on polystyrene trays, over-wrapped with polyvinyl chloride (PVC) oxygen-permeable film, and randomly assigned for 0, 3, 6, and 9 days at 4 °C (2 portions/day; 1 for color and texture and 1 for biochemical analyses). The samples were assigned for analyses of meat pH, water holding capacity, instrumental color, and texture profile (hardness, springiness, cohesiveness, and chewiness).

### 2.2.1 Meat pH

The pH was measured utilizing a digital bench meter (model PHS- 3E-BI, Satra, Kettering, United Kingdom) following the method described by AOAC (2012).

### 2.2.2 Water Holding Capacity (WHC)

The WHC was evaluated according to Quéguiner et al. (1989) and Verbeken et al. (2005). Samples (10g) were centrifuged ( $12\ 000 \times g / 30\ \text{minutes} / 4\ ^\circ\text{C}$ ), the supernatant was discarded, and the tubes with the sample were reweighed. The results were estimated as a percentage of water retention using the following formula:

$$\text{WHC} = (\text{W2}/\text{W1}) \times 100$$

Where: W1 = weight of samples before centrifugation; W2 = weight of the sample after centrifugation.

### 2.2.3 Instrumental Color Evaluation

*Commission Internationale de l'Eclairage* for calculating CIE  $L^*a^*b^*$  color parameters was obtained. The surface values of lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) of pirarucu bellies were analyzed according to AMSA (2012) using a portable spectrophotometer (CM-600D, Konica Minolta Sensing Inc., Osaka, Japan) with 8 mm aperture, illuminant A, and standard 10° observer. The color was analyzed in three different locations on the sample surface, and the color stability was estimated through the calculation of reflectance ratio at 630nm and 580nm (R630/580) according to AMSA (2012).

### 2.2.4 Texture Profile Analysis

The texture profile analysis (TPA) was evaluated according to Huidobro et al. (2005) using a TA.XT Plus texture analyzer (Stable Micro System, United Kingdom) with a cylindrical metal probe of 72 mm diameter. Three cubes of 1.0 cm x 1.0 cm x 1.0 cm were obtained from each sample and subjected to a compression of 75% of the weight in three cycles: pre-test (3 mm/s), test (1 mm/s), and post-test (3 mm/s), with an interval of two seconds between compressions. The Exponent texture software (Stable Micro System, Godalming, United Kingdom) was used to express the results as hardness, springiness, cohesiveness, and chewiness.

### 2.2.5 Statistical Analysis

This study utilized ten ( $n = 10$ ) bellies of pirarucu. Two-way ANOVA (XLSTAT software; Version 2014.5.03, Addinsoft, Inc., Brooklyn, USA) was utilized for analyses of meat pH, water holding capacity, instrumental color, and texture profile to evaluate the effect of bleeding and days of storage (0, 3, 6 and 9 days). Tukey's test was performed to compare treatment means (5% significance level;  $P < 0.05$ ).

## 3. Results

### 3.1 Meat pH

There was no storage x bleeding interaction ( $P = 0.097$ ; Figure 1). However, there was an effect of storage on pH ( $P = 0.000$ ). Regarding treatment, BLE and NON-BLE bellies exhibited similar ( $P > 0.05$ ) pH values on days 0, 3, 6, and 9 (Figure 1). BLE samples demonstrated an increase ( $P < 0.05$ ) in pH values from day 6 of storage, whereas their NON-BLE counterparts exhibited a pH increase ( $P < 0.05$ ) on day 9.

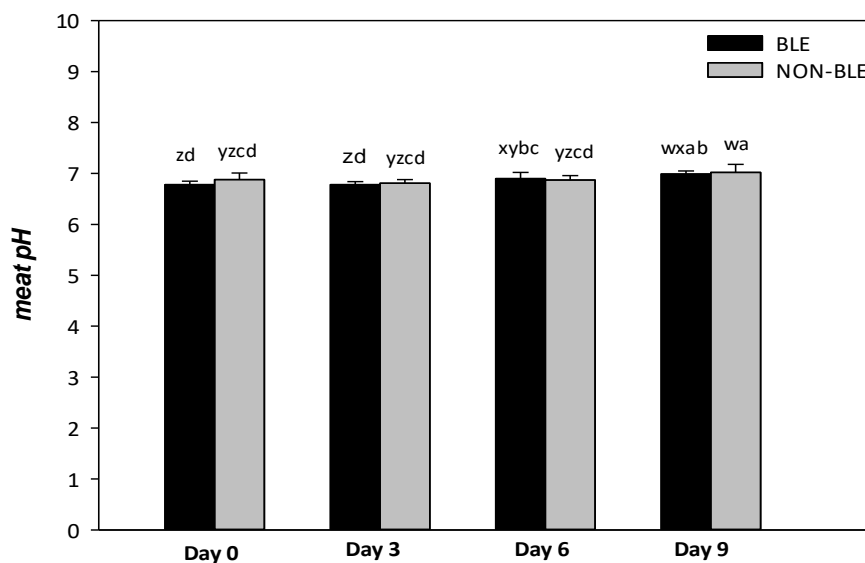


Figure 1. Meat pH of pirarucu bellies harvested with bleeding (BLE) or without bleeding (NON-BLE) during aerobic storage at 4 °C for 9 days. Standard error bars are indicated. <sup>a-c</sup> Means within a harvest method without common superscripts are different ( $P < 0.05$ ). <sup>x,y</sup> Means within a day of storage without common superscripts are different ( $P < 0.05$ ).

### 3.2 Water Holding Capacity (WHC)

There was no storage x bleeding interaction ( $P = 0.288$ ; Figure 2) on WHC. However, there was a storage effect ( $P = 0.000$ ) on WHC. Between treatments, BLE and NON-BLE bellies exhibited similar ( $P > 0.05$ ) WHC values on days 0, 3, 6, and 9 (Figure 2). During storage, BLE and NON-BLE samples demonstrated similar ( $P > 0.05$ ) WHC from day 0 to 9.

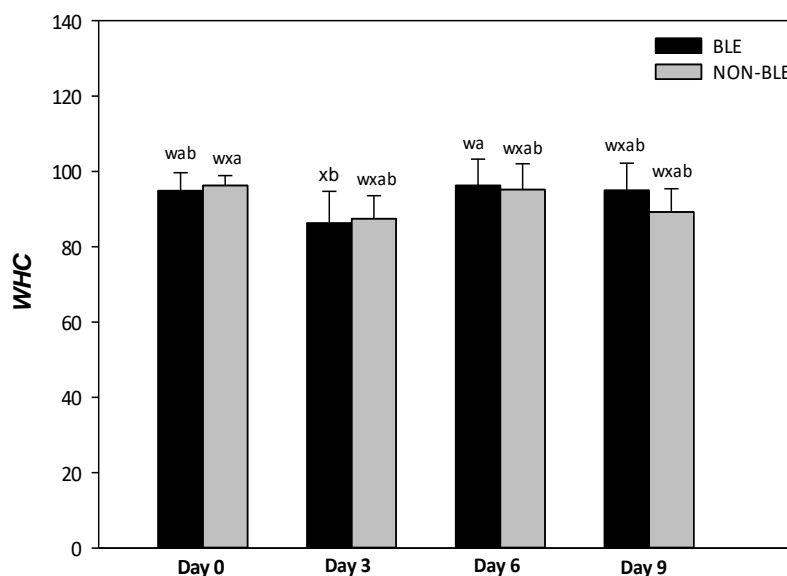


Figure 2. Water holding capacity of pirarucu bellies harvested with bleeding (BLE) or without bleeding (NON-BLE) during aerobic storage at 4 °C for 9 days. Standard error bars are indicated. <sup>a-c</sup> Means within a harvest method without common superscripts are different ( $P < 0.05$ ). <sup>x,y</sup> Means within a day of storage without common superscripts are different ( $P < 0.05$ ).

### 3.3 Instrumental Color

#### 3.3.1 Lightness ( $L^*$ values)

Regarding lightness ( $L^*$  value) there was no storage x bleeding interaction ( $P = 0.304$ ; Figure 3). However, there was effect of bleeding ( $P = 0.018$ ) and storage ( $P = 0.013$ ) for  $L^*$  values. Between treatments, BLE and NON-BLE bellies exhibited similar ( $P > 0.05$ ) lightness on days 0, 3, 6, and 9 (Figure 3). During storage, BLE and NON-BLE samples exhibited similar ( $P > 0.05$ )  $L^*$  values from day 0 to 9.

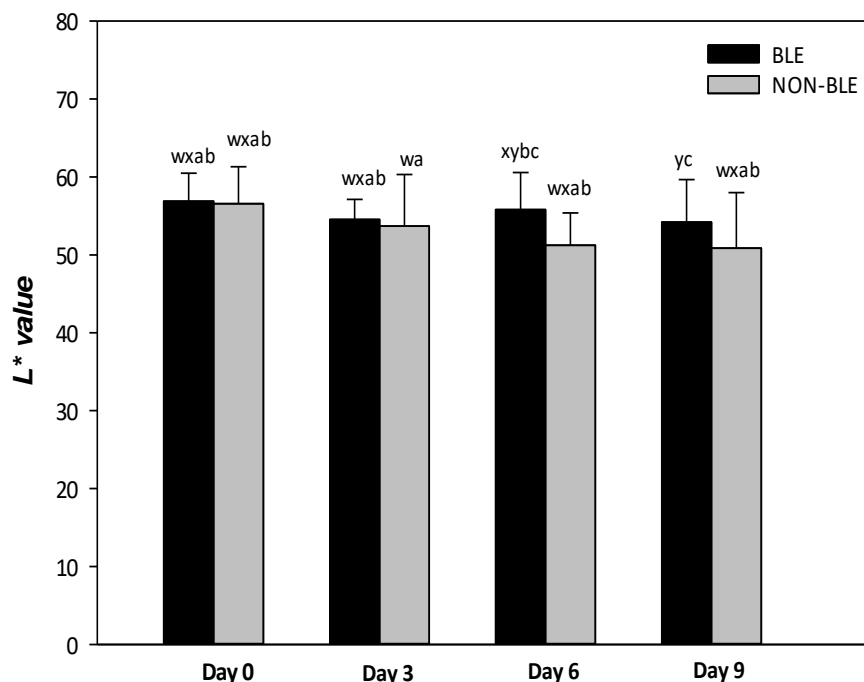


Figure 3. Surface lightness ( $L^*$  values) of pirarucu bellies harvested with bleeding (BLE) or without bleeding (NON-BLE) during aerobic storage at 4 °C for 9 days. Standard error bars are indicated. <sup>a-c</sup> Means within a harvest method without common superscripts are different ( $P < 0.05$ ). <sup>x,y</sup> Means within a day of storage without common superscripts are different ( $P < 0.05$ ).

#### 3.3.2 Redness ( $a^*$ values)

There was a storage x bleeding interaction ( $P = 0.001$ ; Figure 4) for redness. Concerning treatment, NON-BLE samples exhibited greater ( $P < 0.05$ )  $a^*$  values than their BLE counterparts on day 3, whereas BLE and NON-BLE exhibited similar ( $P > 0.05$ ) redness on days 0, 6, and 9 (Figure 4). Throughout storage, both BLE and NON-BLE bellies demonstrated similar ( $P > 0.05$ ) redness.

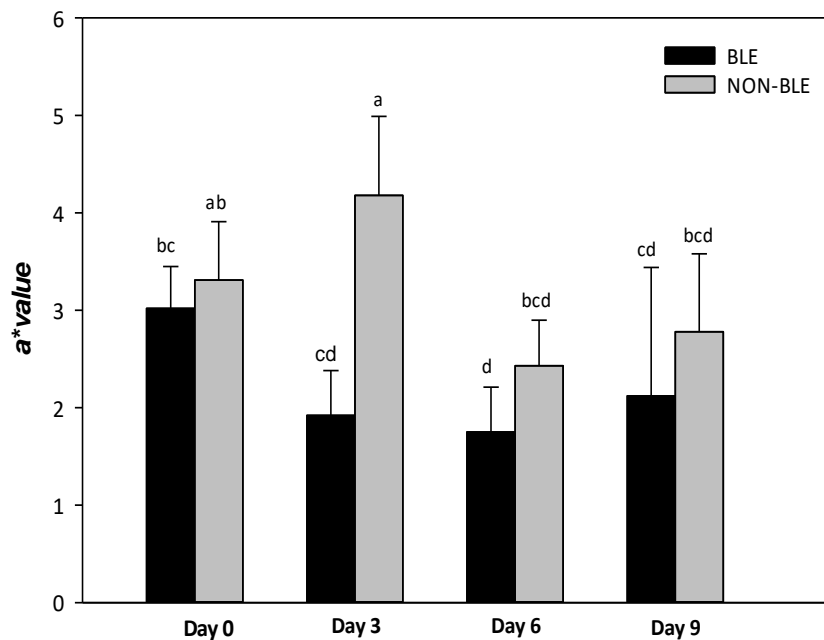


Figure 4. Surface redness ( $a^*$  values) of pirarucu bellies harvested with bleeding (BLE) or without bleeding (NON-BLE) during aerobic storage at 4 °C for 9 days. Standard error bars are indicated. <sup>a-c</sup> Means within a harvest method without common superscripts are different ( $P < 0.05$ ). <sup>x,y</sup> Means within a day of storage without common superscripts are different ( $P < 0.05$ ).

### 3.3.3 Yellowness ( $b^*$ values)

Regarding yellowness ( $b^*$  value), there was storage x bleeding interaction ( $P = 0.001$ ; Figure 5). Between treatments, NON-BLE samples exhibited greater ( $P < 0.05$ )  $b^*$  values than their BLE counterparts on days 3 and 9, whereas BLE and NON-BLE exhibited similar ( $P > 0.05$ ) yellowness on days 0 and 6 (Figure 5). Storage did not ( $P > 0.05$ ) affect the yellowness of NON-BLE samples; however, BLE bellies demonstrated a decrease ( $P < 0.05$ ) in  $b^*$  values on day 9.



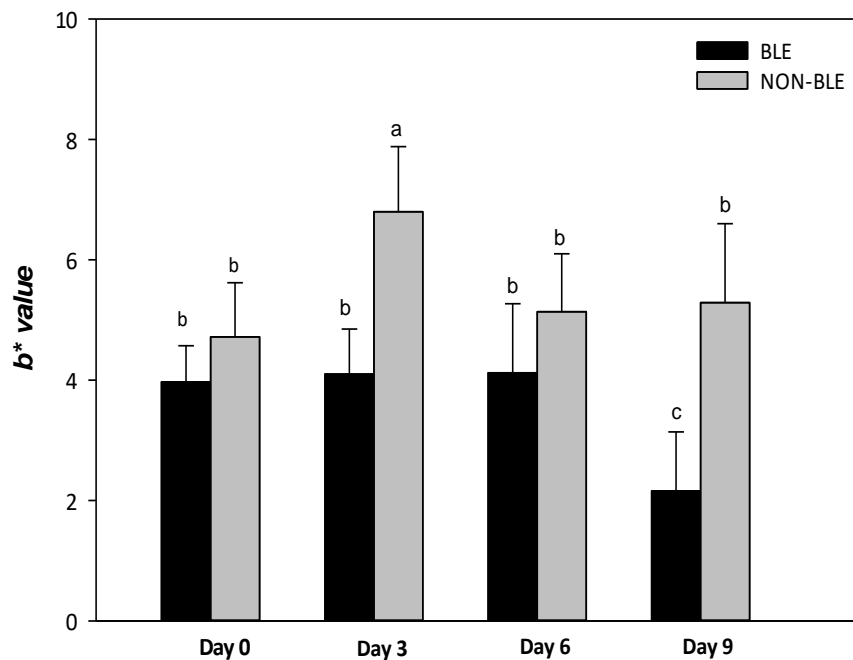


Figure 5. Surface yellowness ( $b^*$  values) of pirarucu bellies harvested with bleeding (BLE) or without bleeding (NON-BLE) during aerobic storage at 4 °C for 9 days. Standard error bars are indicated. <sup>a-c</sup> Means within a harvest method without common superscripts are different ( $P < 0.05$ ). <sup>x,y</sup> Means within a day of storage without common superscripts are different ( $P < 0.05$ ).

### 3.3.4 Color Stability (R630/580)

There was no storage x bleeding interaction ( $P = 0.067$ ; Figure 6) on color stability (R630/580). In addition, there was effect of bleeding ( $P = 0.000$ ) and storage ( $P = 0.001$ ) on R630/580. Regarding treatment, NON-BLE samples exhibited greater ( $P < 0.05$ ) color stability (R630/580) than their BLE counterparts on day 9, whereas BLE and NON-BLE exhibited similar ( $P > 0.05$ ) color stability on days 0, 3, and 6 (Figure 6). BLE bellies exhibited a decrease ( $P < 0.05$ ) in color stability (R630/580) from day 0 to 9 of storage, whereas the values of R630/580 in their NON-BLE counterparts remained stable ( $P > 0.05$ ) throughout storage.

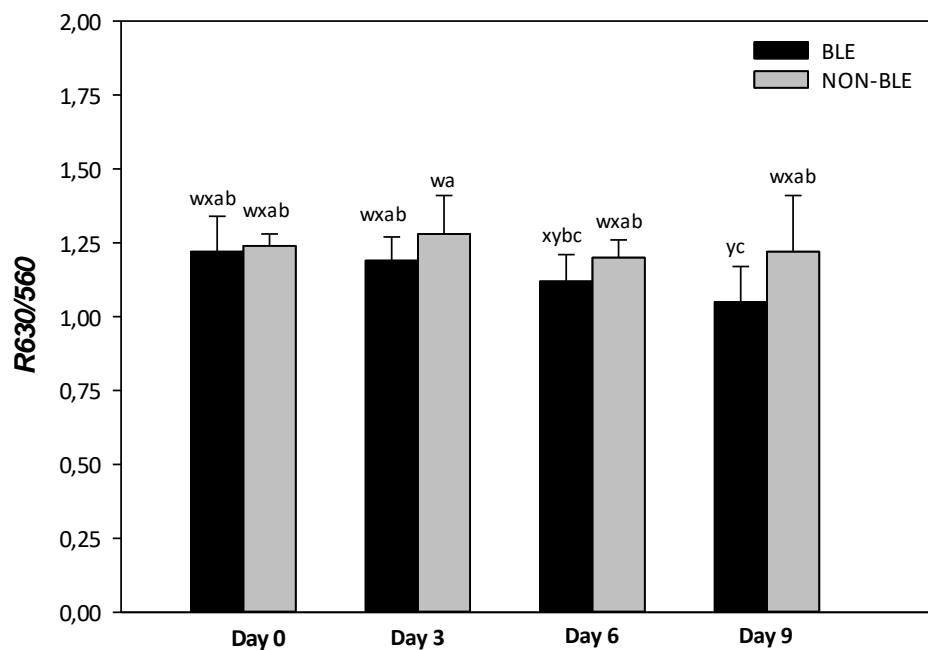


Figure 6. Color stability (R630/580) of pirarucu bellies harvested with bleeding (BLE) or without bleeding (NON-BLE) during aerobic storage at 4 °C for 9 days. Standard error bars are indicated. <sup>a-c</sup> Means within a harvest method without common superscripts are different (P < 0.05). <sup>x,y</sup> Means within a day of storage without common superscripts are different (P < 0.05).

### 3.4 Texture Profile

#### 3.4.1 Hardness

For hardness, there was storage x bleeding interaction (P = 0.000; Table 1). Concerning treatment, BLE bellies exhibited greater (P < 0.05) hardness than their NON-BLE counterparts on day 9, whereas BLE and NON-BLE exhibited similar (P > 0.05) hardness on days 0, 3, and 6 (Table 1). While storage did not influence the hardness of NON-BLE samples, their BLE counterparts demonstrated an increase (P < 0.05) of hardness from day 0 to 9 of storage.

Table 1. Hardness, chewiness, springiness, and cohesiveness of bellies from *Arapaima gigas* harvested with bleeding (BLE) or without bleeding (NON-BLE) during aerobic storage at 4°C for 9 days.

Parameter	Treatment	Days of storage			
		0	3	6	9
Hardness (N) <sup>Δ</sup>	BLE	1.66 ± 0.50 <sup>d</sup>	2.24 ± 0.64 <sup>cd</sup>	4.09 ± 1.56 <sup>ab</sup>	5.48 ± 1.90 <sup>a</sup>
	NON-BLE	2.19 ± 0.61 <sup>cd</sup>	2.30 ± 0.46 <sup>cd</sup>	3.22 ± 0.53 <sup>bc</sup>	2.70 ± 0.52 <sup>bcd</sup>
Chewiness (N) <sup>Δ</sup>	BLE	0.49 ± 0.09 <sup>b</sup>	0.53 ± 0.08 <sup>b</sup>	2.28 ± 0.74 <sup>a</sup>	2.20 ± 0.99 <sup>a</sup>
	NON-BLE	0.50 ± 0.09 <sup>b</sup>	0.65 ± 0.10 <sup>b</sup>	1.16 ± 0.22 <sup>b</sup>	0.89 ± 0.13 <sup>b</sup>
Springiness <sup>Ω</sup>	BLE	0.56 ± 0.11 <sup>wa</sup>	0.59 ± 0.10 <sup>wa</sup>	0.64 ± 0.11 <sup>wa</sup>	0.62 ± 0.07 <sup>wa</sup>
	NON-BLE	0.58 ± 0.12 <sup>wa</sup>	0.55 ± 0.09 <sup>wa</sup>	0.66 ± 0.10 <sup>wa</sup>	0.61 ± 0.09 <sup>wa</sup>
Cohesiveness <sup>Ω</sup>	BLE	0.54 ± 0.05 <sup>wa</sup>	0.53 ± 0.04 <sup>wa</sup>	0.55 ± 0.07 <sup>wa</sup>	0.57 ± 0.06 <sup>wa</sup>
	NON-BLE	0.52 ± 0.06 <sup>wa</sup>	0.51 ± 0.07 <sup>wa</sup>	0.56 ± 0.07 <sup>wa</sup>	0.52 ± 0.06 <sup>wa</sup>

Results are expressed as average  $\pm$  standard deviation (SD).

<sup>Δ</sup> Parameters with storage x bleeding interaction. Therefore <sup>a-d</sup> means without common superscripts are different ( $P < 0.05$ ).

<sup>Ω</sup> Parameters without storage x bleeding interaction. Therefore <sup>a-d</sup> means without common superscripts in a row are different ( $P < 0.05$ ) and

<sup>w-z</sup> means without common superscripts in a column within an attribute are different ( $P < 0.05$ ).

(N) Newton

### 3.4.2 Chewiness

Regarding chewiness, there was storage x bleeding interaction ( $P = 0.000$ ; Table 1). Between treatments, BLE bellies exhibited greater ( $P < 0.05$ ) chewiness than their NON-BLE bellies on days 6 and 9. During storage, BLE samples demonstrated an increase ( $P < 0.05$ ) of chewiness from day 0 to 9, whereas NON-BLE exhibited stable values ( $P > 0.05$ ) throughout the storage.

### 3.4.3 Springiness

There was no storage x bleeding interaction ( $P = 0.551$  Table 1) for springiness. However, there was an effect of storage ( $P = 0.010$ ) on springiness. Regarding treatment, BLE and NON-BLE bellies exhibited similar ( $P > 0.05$ ) springiness on days 0, 3, 6, and 9 (Table 1). Both BLE and NON-BLE samples exhibited similar springiness ( $P > 0.05$ ) throughout the storage.

### 3.4.4 Cohesiveness

For cohesiveness, there was no storage x bleeding interaction ( $P = 0.366$ ; Table 1). However, there was an effect of bleeding ( $P = 0.044$ ) on cohesiveness. For treatment, BLE and NON-BLE bellies exhibited similar ( $P > 0.05$ ) cohesiveness on days 0, 3, 6, and 9 (Table 1). BLE and NON-BLE samples exhibited similar cohesiveness ( $P > 0.05$ ) throughout the storage.

## 4. Discussion

### 4.1 Meat pH

The bleeding did not influence the pH of our samples under refrigeration. The similarity in bellies' pH could be due to normal pre-slaughter stress (Erikson et al., 1999). During harvest, BLE and NON-BLE pirarucus could be subjected to handling stress, affecting the rate of ATP depletion. Immediately after death, the muscle phosphocreatine is completely degraded in stressed fish, and the level of ATP is reduced up to 60%. This in turn, shifts the aerobic to anaerobic metabolism contributing to a decrease in muscle pH (Erikson et al., 1999) in both BLE and NON-BLE samples.

In agreement, Harrysson et al. (2020) found similar pH muscles in bled and un-bled trout

(*Oncorhynchus mykiss*) on days 0, 5, and 10 of storage. Alvarado et al. (2007) evaluated the effect of blood removal on breast chicken and did not find significant differences in pH among different methods of stunning/bleeding and no bleeding. Contrasting our results, Olsen et al. (2006) evaluated the effect of slaughter methods in fillets of Atlantic salmon (*Salmo salar*) with and without bleeding and reported greater pH values in bled samples compared to their un-bled counterparts.

During storage, the increase of pH values in BLE and NON-BLE samples could be attributed to proteolysis and generation of basic compounds, such as amines, in the post-rigor period, contributing to the increase of alkalinity during storage (Duarte et al., 2020).

In agreement with our results, Harrysson et al. (2020) reported a pH increase in bled and un-bled trout (*Oncorhynchus mykiss*) in the first five days of refrigerated storage. Contrasting our results, Sohn et al. (2007) evaluated the effect of bleeding and perfusion on the quality of yellowtail (*Seriola quinqueradiata*) and documented a decrease in pH values in bled and un-bled samples throughout three days of storage.

#### 4.2 Water Holding Capacity

There was no difference in WHC between BLE and NON-BLE samples, probably due to the similar pH (Huff-Lonergan & Lonergan, 2005) of BLE and NON-BLE samples. The pH close to neutrality maintained the net charge of myofibrillar proteins and the muscle cell structure and its components (myofibrils, cytoskeletal linkages, and membrane permeability), contributing to the retention of entrapped water and consequently the maintenance of water holding capacity (Huff-Lonergan & Lonergan, 2005).

In agreement, Digre et al. (2011) observed similar WHC values in Atlantic cod muscle (*Gadus morhua*) subjected to harvest with and without bleeding. In contrast, Rotabakk et al. (2014) studied the effect of blood removal in un-bled (direct gutting) and bled (gill cutting) farmed Atlantic cod (*Gadus morhua*) and documented that both bled and non-bled animals exhibited a decrease in WHC during seven days of storage.

No differences in WHC were observed in BLE and NON-BLE samples during nine days of storage. It could probably be to the similarities in muscle pH of BLE and NON-BLE bellies, contributing to the sustaining of intermolecular protein linkages (Rotabakk et al., 2011) and maintenance of WHC.

Supporting our results, Digre et al. (2011) observed similar WHC values in bled and un-bled Atlantic cod (*Gadus morhua*) throughout seven days of storage. In contrast, Rotabakk et al. (2011) reported a decrease in WHC in Atlantic cod (*Gadus morhua*) samples throughout seven days of iced storage.

#### 4.3 Instrumental Color

##### 4.3.1 Lightness ( $L^*$ values)

Regarding instrumental color, there was no difference in lightness between BLE x NON-BLE on days 0, 3, 6, and 9. The observed similarities may be related to the residual blood in the

carcass (Ramos et al., 2005). The residual blood and consequent presence of heme pigments can contribute to a darker meat surface (Ramos et al., 2005) and similar  $L^*$  values. In agreement, Alvarado et al. (2007) evaluated the effect of blood removal in broiler breast meat and reported similar lightness ( $L^*$  values) between bled and un-bled animals. In addition, Erikson et al. (2010) evaluated the effect of bleeding in Atlantic salmon (*Salmo salar*). They did not observe significant differences in lightness on fillets of bled and un-bled animals. In contrast, Roth et al. (2005) reported higher  $L^*$  values in bled Atlantic salmon (*Salmo salar*) fillets compared to their un-bled counterparts after 12 hours under refrigeration (4 °C).

During storage time, no difference in lightness was observed in BLE and NON-BLE from day 0 to 9. The pH maintenance may have influenced the WHC and, consequently, the superficial light scattering (Hughes et al., 2017), maintaining  $L^*$  values during storage. In agreement with our results, Sterniša et al. (2018) documented similar  $L^*$  values in bled carp (*Cyprinus carpio*) fillets compared to un-bled carp during 12 days of refrigerated storage (4 °C). In contrast, Addeen et al. (2014) reported a decrease of lightness in the bled and un-bled chicken breast during eight days of refrigerated storage.

#### 4.3.2 Redness ( $a^*$ values)

Concerning redness, NON-BLE bellies exhibited greater redness than their BLE counterparts on day 3. This result could be attributed to the maintenance of heme pigments such as hemoglobin and myoglobin in muscle (Richards & Hultin, 2002), contributing to the increase of  $a^*$  values in NON-BLE samples. In agreement, Sterniša et al. (2018) observed that un-bled carp (*Cyprinus carpio*) fillets exhibited greater  $a^*$  values than bled carp on the third day of storage under refrigerated storage. Jiang et al. (2019) studied the effect of blood deposition on the flesh quality of yellowtail (*Seriola quinqueradiata*). They reported higher redness in un-bled yellowtail muscle on day 3 of the storage (4 °C). In contrast, Erikson et al. (2010) evaluated the effect of bleeding in Atlantic salmon (*Salmo salar*) fillets and reported similar  $a^*$  values in un-bled and bled samples.

BLE and NON-BLE bellies exhibited similar redness from day 0 to 9 of refrigerated storage. The observed results may be attributed to muscle pH (Ramanathan & Mancini, 2018). Muscle pH can affect color by influencing oxygen consumption and metmyoglobin reducing activity (Ramanathan & Mancini, 2018). The pH values near neutrality enhance the capacity of mitochondria to compete with the myoglobin for available oxygen, resulting in more metmyoglobin formation (Seyfert et al., 2006). In partial agreement, Alvarado et al. (2007) reported similar  $a^*$  values in broiler breast meat from bled and un-bled animals. In contrast, Sterniša et al. (2018) reported an increase of  $a^*$  values in fillets of bled and un-bled common carp (*Cyprinus carpio*) during 12 days of storage.

#### 4.3.3 Yellowness ( $b^*$ values)

Regarding yellowness,  $b^*$  values were greater in NON-BLE than in BLE counterparts on days 3 and 9 of storage. Similar to redness, greater  $b^*$  values could be attributed to the presence of heme pigments (hemoglobin and myoglobin) in NON-BLE samples (Richards & Hultin, 2002) and the high content of polyunsaturated fatty acids in pirarucu's bellies

(Pino-Hernández et al., 2020). This in turn, can favor the myoglobin oxidation induced by lipid oxidation (Faustman et al., 2010) and accumulation of metmyoglobin onto bellies surfaces (Wei et al., 2017), contributing to the increase of  $b^*$  values on NON-BLE at days 3 and 9.

In partial agreement, Erikson et al. (2010) evaluated the effect of bleeding in Atlantic salmon (*Salmo salar*) fillets and reported similar  $b^*$  values in un-bled and bled samples. On contrary, Ramos et al. (2005) evaluated meat color and pigment levels in bled and un-bled bullfrogs (*Rana catesbeiana*) and reported greater  $b^*$  values on bled samples.

During storage, a decrease in yellowness ( $b^*$  values) was observed in BLE bellies, whereas in NON-BLE counterparts,  $b^*$  values remain stable. Losses of yellowness could be attributed to lipid oxidation and changes in heme pigments (Adeen et al., 2017), decreasing yellowness. In contrast, Sterniša et al. (2018) documented greater yellowness in fillets of un-bled common carp (*Cyprinus carpio*) throughout 12 days of storage. Nguyen & Phan (2018) reported greater  $b^*$  values in un-bled cobia (*Rachycentron canadum*) fillets compared to bled samples throughout 24 weeks of storage. Moreover, Digre et al. (2011) reported an increase of  $b^*$  values in bled and un-bled Atlantic cod (*Gadus morhua*) fillets after seven days of iced storage. Sterniša et al. (2018) documented greater yellowness in fillets of un-bled common carp (*Cyprinus carpio*) throughout 12 days of storage.

#### 4.3.4 Color Stability (R630/580)

Regarding R630/580, a higher ratio was observed in NON-BLE when compared to BLE bellies on day 9 of storage. Greater color stability in NON-BLE may be due to the absence to contact of with oxygen in un-bled samples. Oxygen acts as a prooxidant and catalytic agent, interacting with unsaturated fatty acids and triggering lipid and myoglobin oxidation (McClements & Decker, 2000). In partial agreement, Ramos et al. (2005) reported a higher accumulation of metmyoglobin and oxymyoglobin in bled bullfrog (*Rana catesbeiana*) than in un-bled counterparts. In addition, Terayma & Yamanaka (2000) evaluated the effects of bleeding on the quality of Skipjack tuna (*Katsuwonus pelamis*) and reported a greater metmyoglobin ratio in un-bled animals than their bled counterparts.

During the storage, BLE samples exhibited a decrease on R630/580 from day 0 to 9, whereas in their NON-BLE counterparts, the color stability remained stable. The R630/580 ratio is used to measure the surface color stability of fresh meat (AMSA, 2012). Myoglobin autoxidation involves the conversion of ferrous oxymyoglobin ( $Fe^{2+}$ ) to iron metmyoglobin ( $Fe^{3+}$ ), resulting in meat discoloration due to metmyoglobin accumulation (Wu et al., 2015). In addition, lipid oxidation produces reactive oxygen species that can impact the redox stability of myoglobin, predisposing it to further oxidation (Wang et al., 2018). According to our results, Sohn et al. (2007) reported similar metmyoglobin concentrations between bled and un-bled yellowtail (*Seriola quinquediata*) muscles during three days of ice storage. On contrary, Huang et al. (2021) found higher metmyoglobin values in un-bled American catfish (*Ictalurus punctatus*).

#### 4.4 Texture Profile

##### 4.4.1 Hardness

BLE bellies exhibited greater hardness than the NON-BLE bellies on day 9 of storage, which could be due to protein oxidation and the formation of protein cross-links in the muscle (Wang et al., 2017). In partial agreement, Addeen et al. (2017) studied the influence of different slaughter methods (bled and un-bled) on chicken texture and observed lower hardness in un-bled samples compared to their bled counterparts during 12 days of storage. Contrasting our results, Rotabakk et al. (2014) observed similar hardness in bled and un-bled farmed Atlantic cod (*Gadus morhua*) during seven days of storage.

During storage, BLE bellies demonstrated an increase in hardness from day 0 to 9 than in NON-BLE bellies, which could be attributed to protein oxidation (Wang et al., 2017). Amino acid side chains in myofibrillar proteins undergo modifications due to oxidative stress, leading to muscle cross-link formation (Bao & Ertbjerg, 2019). This in turn, may have contributed to the increase of hardness in BLE samples. In partial agreement, Rotabakk et al. (2014) reported greater hardness in bled samples compared to un-bled counterparts. In contrast, Addeen et al. (2017) studied the influence of different slaughter methods (bled and un-bled) on the texture of chicken patties and observed a decrease of hardness both (bled and un-bled) during 12 days of storage.

##### 4.4.2 Chewiness

BLE bellies exhibited greater chewiness than NON-BLE bellies on days 6 and 9, which could be possibly attributed to protein oxidation and the formation of protein cross-links in meat (Wang et al., 2017). In partial agreement, Jing et al. (2013) reported greater chewiness in bled tilapia (*Oreochromis* sp.) than their un-bled counterparts on day 6.

During storage, BLE samples demonstrated an increase of chewiness from day 0 to 9, whereas NON-BLE exhibited similar chewiness throughout the storage. In partial agreement, Addeen et al. (2017) observed similar chewiness in chicken patties from bled and un-bled animals. On contrary, Jing et al. (2013) documented a decrease in chewiness in bled and un-bled tilapia (*Oreochromis* sp.) throughout 12 days of iced storage.

##### 4.4.3 Springiness

BLE and NON-BLE exhibited similar springiness on days 0, 3, 6, and 9, probably due to muscle pH (Ruiz-Ramírez et al., 2005). Samples with pH values close to neutrality sustain the intermolecular linkages between negatively and positively charged groups, which would explain the similar springiness observed throughout storage (Ruiz-Ramírez et al., 2005). In partial agreement, Addeen et al. (2017) observed similar springiness in chicken patties from bled and un-bled animals during 12 days of storage. In contrast, Jing et al. (2013) reported greater springiness in bled tilapia (*Oreochromis* sp.) than un-bled counterparts on days 0, 6, 9, and 12 of storage.

BLE and NON-BLE exhibited similar springiness throughout storage. In agreement, Addeen et al. (2017) observed similar springiness in chicken patties from bled and un-bled animals

throughout 12 days of refrigerated storage. On contrary, Jing et al. (2013) reported an increase in springiness on bled tilapia (*Oreochromis* sp.) compared to their un-bled counterparts from day 0 to 4 of storage.

#### 4.4.4 Cohesiveness

Concerning cohesiveness, BLE and NON-BLE bellies exhibited similar cohesiveness on days 0, 3, 6, and 9. The observed similarities may have attributed to the maintenance of protein conformation, collagen linkage, tissue properties, and protein solubility, contributing to the similar cohesiveness in pirarucu's bellies (Hultmann & Hustad, 2002). In agreement, Addeen et al. (2017) observed similar cohesiveness in chicken patties from bled and un-bled animals during 12 days of refrigerated storage. Contrasting our results, Jing et al. (2013) reported greater cohesiveness in bled tilapia (*Oreochromis* sp.) compared to un-bled counterparts during 12 days of storage.

BLE and NON-BLE exhibited similar cohesiveness throughout storage. In agreement, Addeen et al. (2017) observed no differences in the cohesiveness of chicken patties from bled and un-bled animals throughout 12 days of storage. In contrast, Jing et al. (2013) reported a decrease followed by an increase of cohesiveness during 12 days of storage in bled and un-bled tilapia (*Oreochromis* sp.).

The authors would like to call attention to the lack of scientific literature regarding the influence of bleeding on the texture profile of fish. Therefore, other meat matrices were used for comparison purposes, highlighting the importance and innovation of the present study.

## 5. Conclusions

The results indicate that the practice of bleeding influenced the color and texture attributes of bellies from pirarucu (*Arapaima gigas*) under refrigeration. BLE exhibited overall greater color stability and texture profile, while NON-BLE demonstrated higher redness and yellowness, indicating that bleeding could improve the quality of the pirarucu (*Arapaima gigas*) meat. The good fisheries practices managed by local communities include a controlled production according to quality standards, and its benefits contribute to the empowerment of artisanal fisheries management. Therefore, standardized procedures such as duration and position of the fish bleeding are recommended to provide a great bleeding efficiency and avoid possible changes in quality, such as oxidation reactions favored by the maintenance of residual blood.

## Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, for providing the master scholarship (88882.456182/2019-01) to Pamela Esteves Bassil. This work was supported by Memorial Chico Mendes, Associação dos Produtores Rurais de Carauari (ASPROC), Operação Amazônia Nativa (OPAN) and Instituto de Desenvolvimento Sustentável de Mamirauá (IDSMA).

The authors also thank the local communities involved in community-based conservation in the Middle Juruá region and indigenous communities of the Middle Purus, United States



Agency for International Development (USAID), Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis (IBAMA) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

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