Research Article ISSN 2641-4295

Food Science & Nutrition Research

Influence of Bleeding on Color, Texture, and Chemical Quality of Frozen Pirarucu from Sustainable Management in Amazon, Brazil

Teixeira Fernanda N[1], Bassil Pamela E[1], Lima Loíse C.S[2], Sandes Paula H[1], Martins Mayara X[1], Salim Ana Paula A.A[1*], Mano Sérgio B[1], Másrico Eliane T[1], Toledo Bruno S[1], Kurihara Leonardo[3,4], Campos-Silva João Vitor[5,6], Barros Ana Paula C[7], da Costa Antônio A.D[8] and Ferreira Micheli S[1]

[1]*Departamento de Tecnologia de Alimentos, Faculdade de Veterinária, Universidade Federal Fluminense (UFF), Niterói, RJ, 24230-340, Brasil.*

[2]*Instituto de Química, Universidade Federal de Rio de Janeiro (UFRJ), RJ, 21941909, Brasil.*

[3]*Operação Amazônia Nativa (OPAN), AM, 69083-370, Brasil.*

[4]*Universidade Federal de Manaus (UFAM), Manaus, AM, 69067-005, Brasil.*

[5]*Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas (UFAL), Av. Lourival Melo Mota, s/n, CEP: 57072-900, Tabuleiro do Martins, Maceió, AL, Brasil.*

[6]*Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, Ås, Norway.*

[7]*Instituto de Desenvolvimento Sustentável Mamirauá (IDSM), Estrada do Bexiga 2584, CEP: 69553-225, Tefé, AM, Brasil.*

[8]*Memorial Chico Mendes, Manaus, AM, 69054-693, Brasil.*

[*]Correspondence:
Ana Paula Amaral de Alcântara Salim, Departamento de Tecnologia de Alimentos, Universidade Federal Fluminense, Avenida Almirante Ary Parreiras, 507, Niterói, RJ 24220-000, Brasil, Tel: +55-21-99879-5978

Received: 22 Jun 2022; **Accepted:** 08 Aug 2022; **Published:** 13 Aug 2022

Citation: Fernanda TN, Pamela BE, Loíse LCS, et al. Influence of Bleeding on Color, Texture, and Chemical Quality of Frozen Pirarucu from Sustainable Management in Amazon, Brazil. Food Sci Nutr Res. 2022; 5(1): 1-11.

ABSTRACT

The influence of bleeding on color, texture profile, and chemical quality of frozen pirarucu loins were investigated. The loins were obtained from eighteen (n=18) pirarucu carcasses from animals slaughtered by bleeding (BLE; n = 9) or without bleeding (NON-BLE; n = 9). The loins were sliced and assigned randomly for 1 and 12 months at -20°C to analyze pH, water holding capacity, instrumental color, and texture profile. BLE samples exhibited higher L values ($P < 0.05$), whereas BLE and NON-BLE exhibited similar ($P > 0.05$) pH, a* values, b* values, and color stability at months 1 and 12. NON-BLE samples demonstrated greater ($P < 0.05$) hardness and chewiness at month 1, whereas similar ($P > 0.05$) springiness and cohesiveness were observed in BLE and NON-BLE samples at months 1 and 12. During storage, Both samples (BLE and NON-BLE) demonstrated a decrease ($P < 0.05$) in pH and water holding capacity; and an increase ($P < 0.05$) in lightness, hardness, and chewiness. Also, BLE and NON-BLE exhibited stable ($P > 0.05$) redness, yellowness, color stability, and cohesiveness. NON-BLE samples exhibited a decrease ($P < 0.05$) in springiness from the 1st to the 12th month of frozen storage. Bleeding positively influenced the quality of frozen pirarucu.*

Keywords
Community-based management, Purus River, Instrumental color and texture, Oxidative stability.

Introduction
The pirarucu (*Arapaima gigas*) is a fish from the Amazon basin, recognized worldwide as the largest continental water fish [1]. Pirarucus consumption is highly accepted due to its large size, muscle yield, white muscle, palatability, low-fat content, and absence of intramuscular bones in muscle cuts [2].

Brazilian production of *A. gigas* was approximately 1,500 tons in 2020, of which 75.61% were obtained from the North region [3]. Regarding exportation, nearly 20,000 units of pirarucu have been exported from managed areas, in Brazil, to the US, since 2017, valuing the arapaima production chain [4].

Amazonian freshwater environments are one of the most threatened ecosystems due to the overexploitation of fish such as pirarucu, a target fish species of marked importance. Community-based management has been developed to protect lakes and fish practices and to ensure pirarucu recovery and conservation while generating significant income and food security for local livelihoods across Amazonian floodplains [5].

Arapaima commercial cuts include loin, belly, and tail [6], which quality is influenced by many extrinsic factors, such as harvest and bleeding [7]. During harvest, the practice of bleeding is not common among the fisheries, and its absence could favor oxidative reactions [8] and color deterioration [7], decreasing product economic value and profit [9].

Recently, Bassil et al. [7] evaluated the influence of bleeding on the color and texture profile of refrigerated pirarucu belly and documented overall greater color stability and texture profile in the samples from bled animals. However, the influence of bleeding on the color and texture of frozen pirarucu is yet to be investigated. Therefore, the objective of the present study was to investigate the influence of bleeding on the color, texture profile, and chemical quality of pirarucu loins under frozen storage.

Material and Methods
This work was part of a collective effort coordinated by Collective of Pirarucu to understand the factors influencing the quality of pirarucu's meat commercialized under coordination by Associação dos Produtores Rurais de Carauari (ASPROC). This research was carried out in collaboration with the Universidade Federal Fluminense (UFF) from an extensive survey, which part of the results are being reported in the present study and had the contribution of several institutions.

Experimental Design
The samples were obtained from the sustainable-use reserves of the Purus River. The region is a federally managed Extractive Reserve - RESEX, legally occupied by riverside communities. Annually, the management of pirarucu occurs by the resident communities, based on the number of pirarucus registered at the lake [5]. The pirarucus were harvested at Purus River (Amazon, Brazil) and processed in a commercial facility under the Brazilian Federal Inspection (Manacapuru, Amazon, Brazil). Therefore, institutional animal care and use committee approval was not obtained. For the experimental design (Figure 1), were utilized eighteen pirarucus

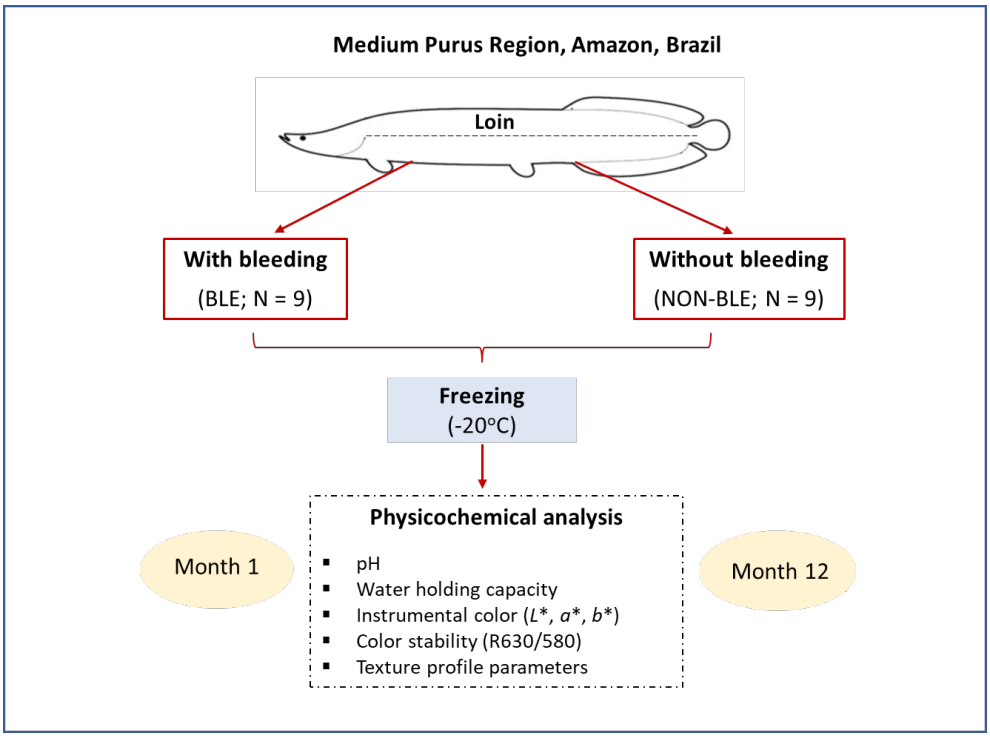

Figure 1: Experimental design.

(A. gigas) harvested either by bleeding (n = 9; BLE) or without bleeding (n = 9; NON-BLE).

The dorsal portion cuts (loin) were obtained, frozen (-20ºC), and shipped to Universidade Federal Fluminense (Niteroi, Rio de Janeiro, Brazil). The samples were assigned randomly for 1 and 12 months to analyze meat pH, water holding capacity (WHC), instrumental color, and texture profile.

pH

The pH was measured utilizing a digital bench meter (model PHS-3E-BI, Satra, Kettering, United Kingdom) according to AOAC [10].

Water Holding Capacity

Water Holding Capacity was estimated according to Quéguiner et al. [11] with modifications proposed by Verbeken et al. [12]. Ten grams of loin samples were centrifuged at 12,000 × g for 30 minutes (4° C). The supernatant was discarded and the tubes with the sample were reweighted. WHC results were estimated as the percentage of water retained according to the following equation:
WHC = (W2 / W1) × 100

Where: W1 represents the weight (g) of the sample before centrifugation and W2 represents the weight (g) of the sample after centrifugation.

Instrumental Color

The surface lightness (*L** values), redness (*a** value) and yellowness (*b** value) of pirarucus loins were analyzed with a portable spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) equipped with illuminant A, 8 mm aperture and standard 10º observer according to AMSA [13]. The color was measured at three random locations on the samples surface and the color stability was estimated through the reflectance ratio at 630nm and 580nm [13].

Texture Profile Analysis (TPA)

The texture profile analysis (TPA) was evaluated according to Huidobro et al. [14], using a texture analyzer (TA.XT Plus; Stable Micro System, United Kingdom) equipped with a cylindrical metal probe (72 mm diameter). Three cubes of 2.0 cm × 2.0 cm × 2.0 cm were obtained from each loin and subjected to compression of 75% of the height 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 Texture Exponent Software (Stable Micro System, United Kingdom) was used to process the data and express the results as hardness, springiness, cohesiveness, and chewiness.

Statistical Analysis

Eighteen (n = 18) loins of pirarucu were utilized in this study. Two-way ANOVA was utilized for analyses of meat pH, water holding capacity, instrumental color, and texture profile to assess the effect of bleeding and months of storage (1 and 12). Tukey's test was used to compare treatment means at 5% significance level ($P < 0.05$). Principal component analysis (PCA) was applied to visualize and interpret the changes in BLE and NON-BLE samples. The results were also evaluated by Pearson's correlation. All analyses were performed using XLSTAT Software (Version 2014.5.03, Addinsoft, Inc., Brooklyn, USA).

Results and Discussion

Muscle pH

There was no bleeding × storage interaction ($P = 0.882$) for pH. However, there was an effect of storage ($P = 0.000$) and bleeding ($P = 0.006$). BLE and NON-BLE exhibited similar pH values ($P > 0.05$) at months 1 and 12 (Figure 2).

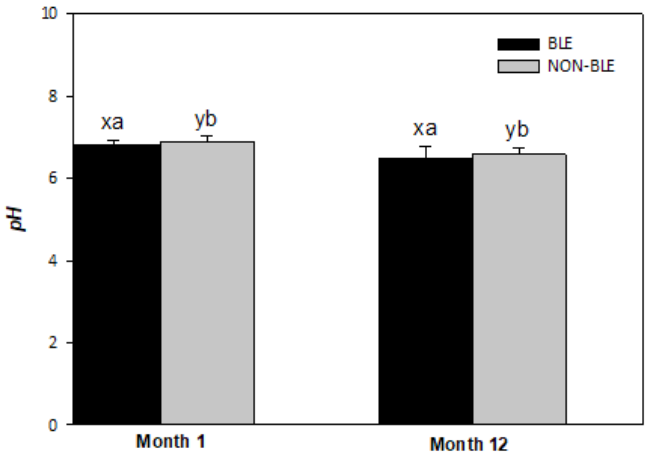

Figure 2: Meat pH of loin obtained from pirarucu (*Arapaima gigas*) harvested with bleeding (BLE) and without bleeding (NON-BLE) on months 1 and 12 of frozen (-20ºC) storage. Means with different letters are different ($P < 0.05$) within harvest method (x-y) and months of storage (a-b). Bars indicate standard deviation.

The similar results observed in muscle pH could be attributed to pre-slaughter handling stress [8]. According to Erikson et al. [8] bled fish is subjected to more handling stress than un-bled counterparts, however both bled and non-bled animals exhibit similar blood glucose and lactate [8]. This in turn may have contributed to the similar pH values observed in BLE and NON-BLE samples.

In partial agreement, Erikson et al. [15] evaluated the influence of bleeding efficiency of anesthetized and exhausted Atlantic salmon (*Salmo salar*) and observed similar pH for both treatments. Alvarado et al. [16] evaluated the influence of stunning methods and bleeding on quality parameters of broiler meat and observed similar pH in meat from bled and un-bled samples after 24 h in refrigerated storage. Digre et al. [17] evaluated the influence of bleeding methods (gill cutting – bled; and direct gutting – un-bled), in quality parameters of cod (*Gadus morhua*) and observed that bled and un-bled fillets exhibited similar pH after 7 days of ice storage. In contrast, Erikson et al. [18] evaluated the influence of live frozen storage (un-bled) and bleeding (bled) on quality parameters of cod (*Gadus morhua*) and observed lower pH in un-bled samples than their bled counterparts.

During storage, BLE and NON-BLE samples demonstrated a decrease ($P < 0.05$) on muscle pH from month 1 to 12. The observed decrease in pH of BLE and NON-BLE samples could be attributed to the glycogen degradation in the postmortem period [19,20]. According to Inohara et al. [20], during the postmortem period, the progression of glycolysis and ATP decomposition are correlated to the physiological state before and after catching and storage temperature. In this sense, the glycolysis continues to progress during frozen storage at -20°C [21], contributing to the decrease of muscle pH in both BLE and NON-BLE.

In partial agreement, Viji et al. [22] evaluated the effect of bleeding on the quality of un-bled (direct gutting) and bled (gill cutting) Catfish Sutchi (*Pangasianodon hipophthalmos*) fillets and reported a reduction of pH in fillets in both samples in the first 5 days of refrigerated storage. On contrary, Sonh et al. [23] evaluated the influence of bleeding in the pH of dark muscles of Yellowtail (*Seriola quinqueradiata*) and documented similar pH in bled and un-bled samples during 48 hours of refrigerated storage.

Water Holding Capacity (WHC)

There was no bleeding × storage interaction ($P = 0.170$) for WHC. However, there was a storage effect ($P = 0.022$) on the WHC. BLE and NON-BLE samples exhibited similar ($P > 0.05$) WHC values on months 1 and 12 of frozen storage (Figure 3), which may be attributed to the similar pH [24] of both samples.

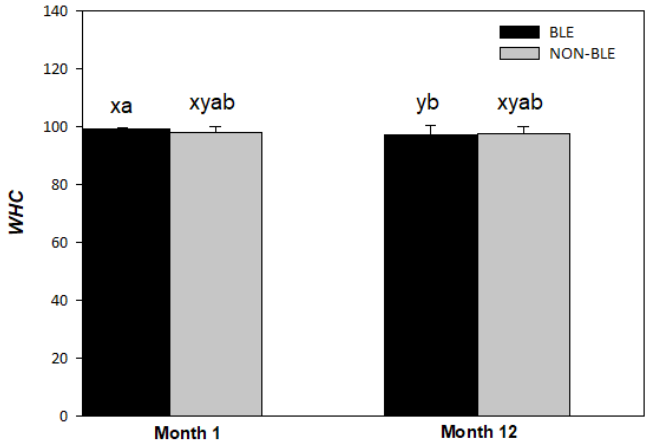

Figure 3: Water holding capacity (WHC) of loin obtained from pirarucu (*Arapaima gigas*) harvested with bleeding (BLE) and without bleeding (NON-BLE) on months 1 and 12 of frozen (-20ºC) storage. Means with different letters are different ($P < 0.05$) within harvest method (x - y) and months of storage (a - b). Bars indicate standard deviation.

Muscle pH close to neutrality kept the net charge of myofibrillar proteins and the structure of the muscle cell, contributing to the retention of entrapped water, and consequently to the maintenance of water holding capacity [24].

In partial agreement, Nguyen and Phan [25] evaluated the influence of bleeding (air bleeding and non-bleeding) on the quality of cobia (*Rachycentron canadum*) and reported similar water content in bled and un-bled samples on months 0 and 6 of frozen storage. Bassil et al. [7] investigated the influence of bleeding on the quality of pirarucu (*Arapaima gigas*) bellies and documented similar WHC in bled and un-bled samples on days 0, 3, 6, and 9 of refrigerated storage.

During storage, BLE samples exhibited a decrease in WHC ($P < 0.05$) from month 1 to 12, whereas in NON-BLE counterparts WHC values remained stable ($P > 0.05$). The observed decrease in WHC of BLE samples could be attributed to protein oxidation [26]. The contact with oxygen during bleeding may have contributed to the increase of protein oxidation, leading to the shrinkage of the interfilamentous spaces and to a loss of water holding capacity. Oxygen acts as a prooxidant and catalytic agent, interacting with unsaturated fatty acids and triggering lipid and protein oxidation [27], contributing to the decrease of WHC [26].

In partial agreement, Nguyen and Phan [25] reported a decrease in water content of bled and un-bled cobia (*Rachycentron canadum*) from 0 to 6 months of frozen storage. Bassil et al. [7] documented similar WHC in bled and un-bled pirarucu (*Arapaima gigas*) bellies during 9 days of refrigerated storage. A positive correlation was observed between WHC and pH ($r = 0.717$; $P < 0.05$), which reiterates the relationship between these parameters.

Instrumental Color

Lightness (*L** values)

There was no bleeding × storage interaction ($P = 0.429$) for lightness. However, there was an effect of bleeding ($P = 0.000$) and storage ($P = 0.000$). BLE samples exhibited higher *L** values ($P < 0.05$) than their NON-BLE counterparts at months 1 and 12 of frozen storage (Figure 4A).

The differences in lightness between BLE and NON-BLE could be attributed to the differences in harvest method [28,29]. Harvest with bleeding reduces the amount of residual blood and heme pigments, which contribute to a lighter meat surface [28,19, 29].

In agreement, Nguyen and Phan [25] evaluated the effect of bleeding methods (air bleeding and non-bleeding) on the quality of cobia (*Rachycentron canadum*) fillets during 24 weeks of frozen storage and documented greater lightness (*L** values) in bled samples than those from un-bled samples. In partial agreement, Sterniša et al. [29] evaluated the effect of bleeding on color of common carp (*Cyprinus carpio*) and documented greater *L** values in fillets from bled carp than those from un-bled counterparts during 12 days of refrigerated storage. Erikson et al. [18] evaluated the influence of bleeding on color of Atlantic Cod (*Gadus morhua*) and documented greater lightness in meat from bled cod than those from un-bled counterparts after freezing for 61 days. Contrasting our results, Huang et al. [30] investigated the influence of bleeding on color, myoglobin content, and lipid and protein oxidation of Channel catfish (*Ictalurus punctatus*) and documented similar lightness in bled and un-bled samples after 18h of frozen storage.

Erikson et al. [15] evaluated the effect of bleeding on color of farmed Atlantic salmon (*Salmo salar*) and documented similar *L** in samples from bled and un-bled samples on day 0 of storage.

Storage affected ($P < 0.05$) the *L* * values of the BLE and NON-BLE samples (Figure 4A). BLE and NON-BLE demonstrated an increase ($P < 0.05$) in *L* * values from month 1 to 12. The observed increase in lightness could be attributed to the muscle pH decrease [24]. The pH decline, observed in BLE and NON-BLE samples from month 1 to 12 (Figure 2), contributed to the decrease of WHC and increase of purge loss [24]. This in turn, change the superficial light scattering in muscle [31] and consequently increased the *L** values during storage. A negative correlation was observed between *L** value and pH ($r = -0.971$, $P < 0.05$) which reiterates the relationship between surface lightness and pH (Table 1).

In contrast, Erikson et al. [18] evaluated the influence of bleeding on the color of Atlantic Cod (*Gadus morhua*) and documented similar lightness in meat from bled un-bed cod during 61 days of frozen storage. Nguyen and Phan [25] evaluated the effect of bleeding methods (air bleeding and non-bleeding) on the quality of cobia (*Rachycentron canadum*) fillets and documented a decrease of lightness (*L** values) in both bled un-bled samples during 24 weeks of frozen storage. Sterniša et al. [29] evaluated the effect of bleeding on color of common carp (*Cyprinus carpio*) and documented similar *L** values in fillets from bled and un-bled carp during 12 days of refrigerated storage.

Redness (*a** values)

There was no bleeding × storage interaction ($P = 0.122$) for *a** value. Additionally, there was no effect of bleeding ($P = 0.764$) and storage ($P = 0.374$). BLE and NON-BLE samples exhibited similar *a** values ($P < 0.05$) at months 1 and 12 of storage (Figure 4B). The observed results may be attributed to muscle pH [32]. Muscle pH influences mitochondria oxygen consumption and metmyoglobin reducing activity, influencing the surface redness [32]. The similar pH values, close to neutrality, observed in BLE and NON-BLE (Figure 2) enhance the capacity of mitochondria to compete with myoglobin for available oxygen, resulting in an increase of metmyoglobin formation in both samples, equally influencing *a** values.

In partial agreement, Erikson et al. [15] evaluated the effect of bleeding on color of farmed Atlantic salmon (*Salmo salar*) and documented similar *a** in samples from both bled and un-bled salmon on day 0 of refrigerated storage. Contrasting with our results, Huang et al. [30] investigated the influence of bleeding on color of Channel catfish (*Ictalurus punctatus*) and documented greater redness in un-bled samples than those from bled counterparts on day 0 of frozen storage. In addition, Nguyen and

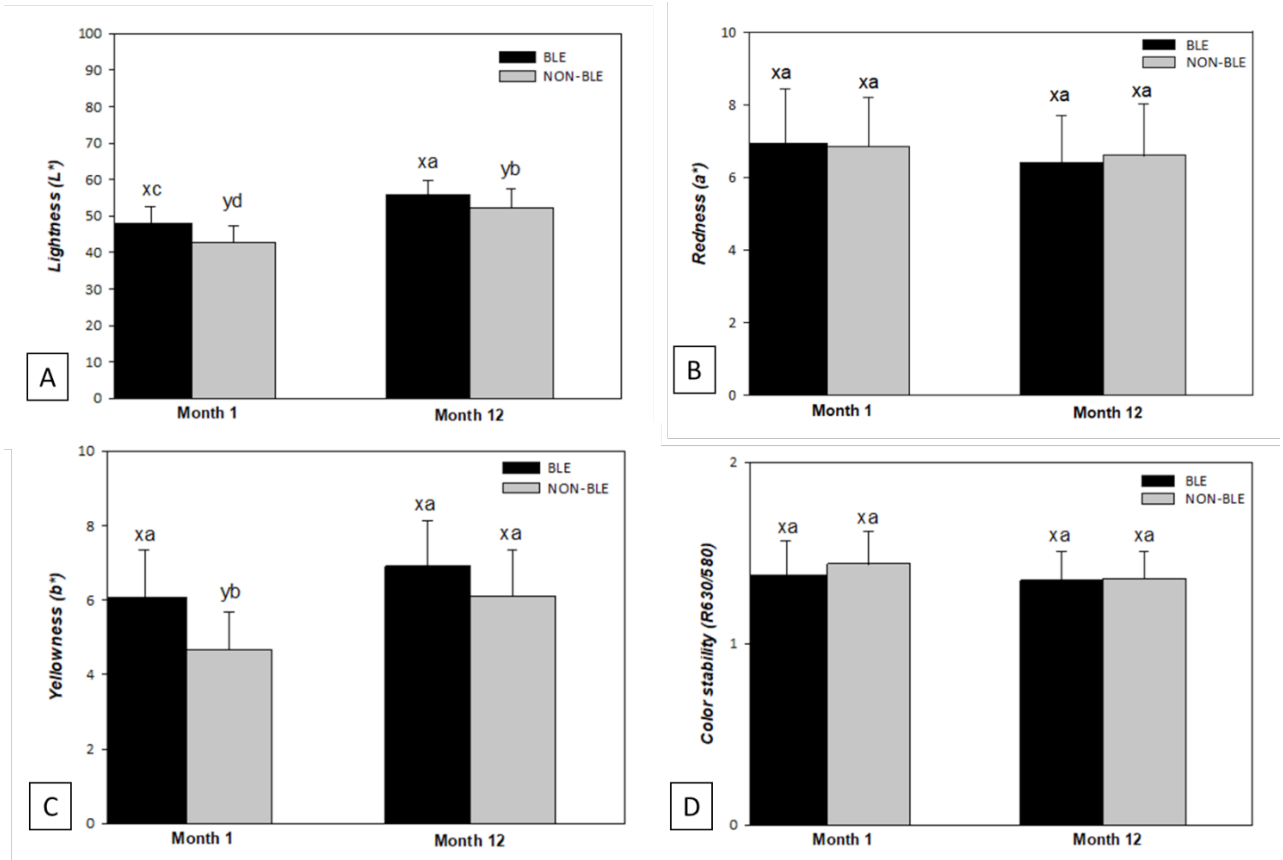

Figure 4: Instrumental color and color stability of loin obtained from pirarucu (*Arapaima gigas*) harvested with bleeding (BLE) and without bleeding (NON-BLE) on months 1 and 12 of frozen (-20ºC) storage. [A] Lightness (*L**), [B] redness (*a**), [C] yellowness (*b**) and [D] color stability (R630/580). Means with different letters are different ($P < 0.05$) within harvest method (x - y) and months of storage (a - d). Bars indicate standard deviation.

Phan [25] evaluated the effect of bleeding methods (air bleeding and non-bleeding) on the quality of cobia (*Rachycentron canadum*) fillets and documented higher redness (*a** values) in un-bled fillets than those from their bled counterparts during 24 weeks of frozen storage. Sterniša et al. [29] evaluated the effect of bleeding on color of common carp (*Cyprinus carpio*) and documented lower *a** values in fillets from bled carp than those from un-bled counterparts during 12 days of refrigerated storage. Furthermore, Erikson et al. [18] evaluated the influence of bleeding on color of Atlantic Cod (*Gadus morhua*) and reported greater redness in meat from un-bled than in their bled counterparts during 61 days of frozen storage.

During storage, both BLE and NON-BLE samples exhibited stable *a** values ($P > 0.05$) from month 1 to 12 of storage (Figure 4B), which could be attributed to the maintenance of heme pigments such as hemoglobin and myoglobin in muscle [33]. Richards and Hultin [33] reported a residual blood and hemoglobin content in bled fish (gill-cut or tail-cut) compared to un-bled control. This fact may occur due to the presence of capillaries surrounding the muscle, which would not empty after incision, especially considering the drop in blood pressure [33]. The quick blood coagulation in fish may also have contributed to the substantial amount of blood in BLE samples [34].

In contrast, Sterniša et al. [29] evaluated the effect of bleeding on the color of common carp (*Cyprinus carpio*) and documented an increase of *a** values in fillets from bled carp than those from un-bled counterparts during 12 days of refrigerated storage. Nguyen and Phan [25] evaluated the effect of bleeding methods (air bleeding and non-bleeding) in cobia (*Rachycentron canadum*) fillets and documented an increase of redness (*a** values) in both bled un-bled samples during 24 weeks of frozen storage. Erikson et al. [18] evaluated the influence of bleeding on color of Atlantic Cod (*Gadus morhua*) and documented a decrease of *a** values in meat from bled and un-bled cod after 61 days of frozen storage.

Yellowness (*b** values)

There was no bleeding × storage interaction ($P = 0.282$) for *b** value. In addition, there was an effect of bleeding ($P = 0.003$) and storage ($P = 0.000$). BLE and NON-BLE samples exhibited similar *b** values ($P > 0.05$) in the 1st and 12th months of storage (Figure 4C). As reported in redness, the similar pH and the presence of heme pigments (hemoglobin and myoglobin) on bled and un-bled samples [33] can equally favor metmyoglobin accumulation onto surface of both samples. This in turn, may have contributed to similar *b** values in both, BLE and NON-BLE. Additionally, *b** values exhibited a positive correlation with pH ($r = 0.956$, $P < 0.05$), and a negative correlation with R630/580 ($r = 0.907$, $P < 0.05$), which further reiterates the relationship between these parameters.

In agreement with our results, Erikson et al. [18] evaluated the influence of bleeding on color of Atlantic Cod (*Gadus morhua*) and documented similar *b** values in meat from both bled and un-bled cod after 61 days of frozen storge. In contrast, Huang et al. [30] investigated the influence of bleeding on color of Channel catfish (*Ictalurus punctatus*) and documented greater yellowness in un-bled samples than those from bled counterparts on day 0 of frozen storage. Nguyen and Phan [25] evaluated the effect of bleeding methods (air bleeding and non-bleeding) on the quality of frozen cobia (*Rachycentron canadum*) and documented higher yellowness (*b** values) in un-bled fillets than those from their bled counterparts during 24 weeks. Sterniša et al. [29] evaluated the effect of bleeding on color of common carp (*Cyprinus carpio*) and documented lower *b** values in fillets from bled carp than those from un-bled counterparts during 12 days of refrigerated storage. Erikson et al. [15] evaluated the effect of bleeding on color of farmed Atlantic salmon (*Salmo salar*) and documented similar *b** in both bled and un-bled samples on day 0 of refrigerated storage.

During storage, BLE and NON-BLE exhibited stable ($P > 0.05$) yellowness from 1 to 12 months of frozen storage (Figure 4C). Contrasting our results, Sterniša et al. [29] documented an increase of *b** values in fillets from bled carp (*Cyprinus carpio*) during 12 days of refrigerated storage. Nguyen and Phan [26] documented an increase of yellowness (*b** values) in bled and un-bled cobia (*Rachycentron canadum*) fillets during 24 weeks of frozen storage. Erikson et al. [18] documented an increase of yellowness (*b** values) in meat from both bled and un-bled Atlantic Cod (*Gadus morhua*) after 61 days of frozen storge.

Color stability (R630/580)

There was no bleeding × storage interaction ($P = 0.493$) for R630/580. Furthermore, there was no effect of bleeding ($P = 0.313$) and storage ($P = 0.124$). BLE and NON-BLE samples exhibited similar R630/580 values ($P > 0.05$) at months 1 and 12 of storage (Figure 4D). R630/580 estimates surface discoloration [13]. High ratios indicate more redness and lower ratios (close to 1) indicate a high content of metmyoglobin and a brownish color [13]. The similar results in R630/580 in both BLE and NON-BLE samples could be attributed to the muscle pH [32] and to the residual blood and heme pigments in muscle [33]. As reported in *a** values, the muscle pH influences mitochondria metabolism and the accumulation of metmyoglobin [32]. Additionally, the presence of iron in myoglobin acts as a prooxidant triggering myoglobin oxidation [27] contributing to the similar values of R630/580 in BLE and NON-BLE. R630/580 exhibited a positive correlation with pH ($r = 0.784$, $P < 0.05$) and a negative correlation with *b** value ($r = 0.907$, $P < 0.05$), reiterating the relationship between this ratio, the content of metmyoglobin and the brownish color represented by *b** values (Table 1).

In partial agreement, Bassil et al. [7] documented greater color stability (R630/580) in bled pirarucu (*A. gigas*) bellies on day 9 of refrigerated storage. Ramos et al. [28] reported a greater accumulation of metmyoglobin in bled bullfrogs (*Rana catesbeiana*) than in their un-bled counterparts. In addition, Terayama and Yamanaka [35] reported a greater metmyoglobin ratio in un-bled Skipjack tuna (*Katsuwonus pelamis*) than in their bled counterparts.

BLE and NON-BLE samples exhibited stable R630/580 values ($P > 0.05$) during 12 months of frozen storage (Figure 4D). In

partial agreement, Sohn et al. [23] reported similar metmyoglobin concentrations in bled and un-bled yellowtail (*Seriola quinquediata*) muscles during 2 days of refrigerated storage. On contrary, Huang et al. [30] reported higher metmyoglobin values in un-bled Channel catfish (*Ictalurus punctatus*). Bassil et al. [7] documented a decrease in color stability (R630/580) in bled bellies from day 0 to 9 of storage, whereas the values of R630/580 in their un-bled counterparts remained stable throughout storage.

Texture Profile

Hardness

There was a bleeding × storage interaction for hardness (P = 0.000). The NON-BLE samples exhibited greater hardness (P < 0.05) than their BLE counterparts at month 1, whereas both BLE and NON-BLE samples exhibited similar hardness (P > 0.05) at month 12 (Figure 5A).

The difference in hardness can be attributed to protein oxidation [36]. Changes in amino acid side chains of myofibrillar proteins are favored by the greater content of myoglobin in un-bled animals, leading to the formation of protein cross-links, contributing to the increase of hardness in NON-BLE samples.

Contrasting our results, Viji et al. [22] evaluated the impact of bleeding on quality of catfish sutchi (*Pangasianodon hipophthalmos*) fillets and reported greater hardness in fillets from bled animals than in those from un-bled counterparts on days 6, 12, 24 and 27 of refrigerated storage. Rotabakk et al. [37] evaluated the influence of blood removal in un-bled (direct gutting) and bled (gill cutting) farmed Atlantic cod (*Gadus morhua*) and documented similar hardness in bled and un-bled samples during 7 days of frozen storage. Furthermore, Addeen et al. [38] evaluated the influence of bleeding on chicken texture and observed greater hardness in bled samples than those from un-bled counterparts during 12 days of refrigerated storage.

Both the BLE and NON-BLE samples exhibited an increase (P < 0.05) in hardness from the 1st to the 12th month of storage (Figure 5A). The observed results can be attributed to protein oxidation and the formation of protein cross-links [36], decreasing the water holding capacity and protein solubility [39] and contributing to the increase of hardness.

In partial agreement, Viji et al. [22] evaluated the impact of bleeding on quality of catfish sutchi (*Pangasianodon hipophthalmos*) fillets and reported an increase of hardness in both bled and un-bled fillets on day 1 of refrigerated storage. Sørensen et al. [40] observed an increase in hardness in bled Atlantic cod (*Gadus morhua*) from month 3 to 12 of frozen storage. In contrast, Rotabakk et al. [37] documented greater hardness in bled samples of farmed Atlantic cod (*Gadus morhua*) compared to un-bled counterparts. Addeen et al. [38] reported a decrease of hardness both bled and un-bled chicken patties during 12 days of storage.

Springiness

There was no bleeding × storage interaction (P = 0.766) on springiness. However, there was a storage effect of storage (P = 0.001). BLE and NON-BLE samples exhibited similar springiness (P > 0.05) at months 1 and 12 of storage (Figure 5B). The observed results could

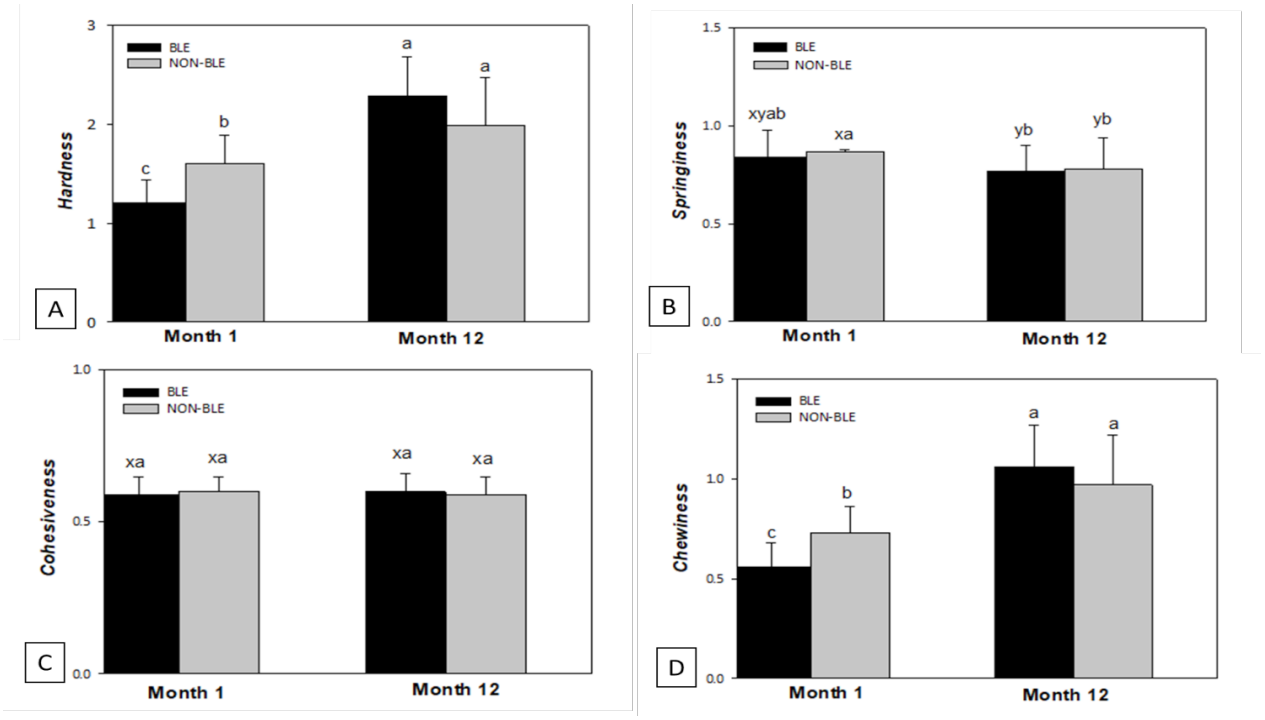

Figure 5: Texture profile of loin obtained from pirarucu (*Arapaima gigas*) harvested with bleeding (BLE) and without bleeding (NON-BLE) on months 1 and 12 of frozen (-20ºC) storage. [A] Hardness, [B] springiness, [C] cohesiveness and [D] chewiness. Means with different letters are different (P < 0.05) within harvest method (x - y) and months of storage (a - d). Parameters with storage x bleeding interaction show only a-c superscripts. Bars indicate standard deviation.

Table 1: Correlation matrix (Pearson test) of pH, WHC, *L*, a* and b*,* R630/580, hardness, cohesiveness, springiness and chewiness of loin obtained from pirarucu (*Arapaima gigas*) harvested with bleeding (BLE) and without bleeding (NON-BLE) and stored frozen (-20◦C) for 1 and 12 months.

Variables	pH	CRA	L*	a*	b*	R630/580	Hardness	Springiness	Cohesiveness	Chewiness
pH	**1**	0.717	**-0.971**	0.355	**-0.956**	0.784	-0.821	**0.992**	-0.163	-0.689
CRA		**1**	-0.529	-0.088	-0.527	0.134	**-0.960**	0.681	-0.626	**-0.994**
*L**			**1**	-0.447	**0.986**	-0.909	0.666	**-0.970**	-0.004	0.495
*a**				**1**	-0.304	0.500	-0.192	0.470	0.829	0.023
*b**					**1**	-0.907	0.627	-0.936	0.113	0.477
R630/580						**1**	-0.297	0.791	0.253	-0.090
Hardness							**1**	-0.817	0.387	**0.969**
Springiness								**1**	-0.050	-0.666
Cohesiveness									**1**	0.565
Chewiness										**1**

be attributed to muscle pH [41]. Samples with pH values close to neutrality maintain the intermolecular linkages between charged groups [41], and water holding capacity [24], which may have contributed to the similar springiness observed throughout storage [41]. Springiness exhibited a strong positive correlation with pH ($r = 0.992$ $P < 0.05$) and WHC ($r = 0.681$ $P < 0.05$) supporting the relationship between these parameters (Table 1).

In partial agreement, Addeen et al. [38] observed similar springiness in chicken patties from bled and un-bled animals during 12 days of storage. In contrast, Jing et al. [42] reported greater springiness in bled tilapia (*Oreochromis* sp.) compared to un-bled counterparts during 12 days of storage. Viji et al. [22] reported greater springiness on bled catfish sutchi (*Pangasianodon hipophthalmos*) fillets than their un-bled counterparts from day 12 to 30 of refrigerated storage.

NON-BLE samples exhibited a decrease ($P<0.05$) in springiness from the 1st to the 12th month of frozen storage (Figure 5B), which could be attributed to the formation of ice crystals and protein oxidation, leading to a decrease in water holding capacity during storage [39]. During frozen storage, the formation of ice crystals promotes mechanical disruption of muscle tissue with release of endogenous proteases, leading to the loss of protein three-dimensional structure [39] and contributing to the decrease of springiness. Additionally, the change of protein rheological properties is associated with a decrease in water holding capacity. Since the proteins decrease their ability to bind water, the water molecules are retained only by capillary forces [39], influencing springiness.

In partial agreement, Viji et al. [22] reported a decrease on springiness in un-bled and bled Catfish Sutchi (*Pangasianodon hipophthalmos*) fillets during 30 days of refrigerated storage. In contrast, Addeen et al. [38] observed similar springiness in chicken patties from bled and un-bled samples throughout 12 days of refrigerated storage. Jing et al. [42] reported an increase of springiness on bled tilapia (*Oreochromis* sp.) compared to their un-bled counterparts from days 0 to 4 of storage.

Cohesiveness

There was no bleeding × storage interaction ($P = 0.202$) for cohesiveness. Furthermore, there was no effect of bleeding ($P = 0.976$) and storage ($P = 0.834$). BLE and NON-BLE samples exhibited similar cohesiveness ($P > 0.05$) at months 1 and 12 of storage (Figure 5C). In agreement, Addeen et al. [38] observed similar cohesiveness in chicken patties from bled and un-bled animals during 12 days of refrigerated storage. Contrasting our results, Jing et al. [42] reported greater cohesiveness in bled tilapia (*Oreochromis* sp.) compared to un-bled counterparts during 12 days of storage. Viji et al. [22] reported a greater cohesiveness in bled (gill cutting) Catfish Sutchi (*Pangasianodon hipophthalmos*) fillets from day 18 to 30 of refrigerated storage. In partial agreement, Bassil et al. [7] documented similar cohesiveness in bled and un-bled pirarucu (*Arapaima gigas*) bellies during 9 days of refrigerated storage.

During storage, the BLE and NON-BLE samples exhibited stable cohesiveness ($P > 0.05$) for 12 months (Figure 4C). In contrast, Jing et al. [42] reported a decrease in cohesiveness in bled and un-bled tilapia (*Oreochromis* sp.) during 12 days of storage. Viji et al. [22] reported a decrease in cohesiveness in bled and un-bled Catfish Sutchi (*Pangasianodon hipophthalmos*) fillets during 30 days of refrigerated storage.

Chewiness

There was an interaction between bleeding × storage for chewiness ($P = 0.003$). Similar to hardness, the NON-BLE samples exhibited greater ($P < 0.05$) chewiness than their BLE counterparts at month 1, whereas BLE and NON-BLE exhibited similar chewiness ($P > 0.05$) in month 12 (Figure 5D). The observed difference in chewiness can be possibly attributed to protein oxidation and the formation of protein cross-links in meat [36], contributing to the increase of chewiness in NON-BLE samples. A positive correlation (Table 1) was observed between chewiness and hardness ($r = 0.969$ $P < 0.05$).

In partial agreement, Jing et al. [42] reported greater chewiness in bled tilapia (*Oreochromis* sp.) than in their un-bled counterparts on day 6 of refrigerated storage. On contrary, Viji et al. [22] reported greater chewiness on bled samples than in their un-bled counterparts from day 6 to 30 of refrigerated storage.

During storage, both BLE and NON-BLE samples exhibited an increase ($P < 0.05$) in chewiness from month 1 to 12 (Figure 5D).

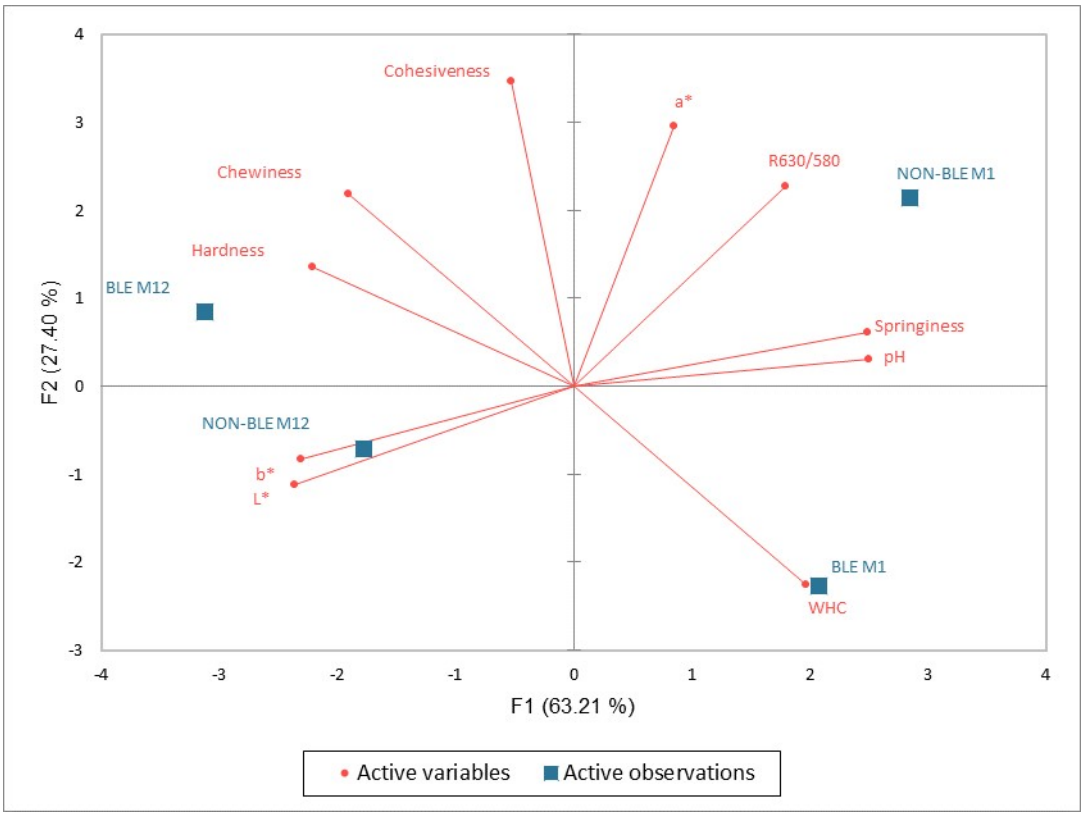

Figure 6: Principal component analysis of instrumental color, texture profile and physical-chemical quality of loin obtained from pirarucu (*Arapaima gigas*) harvested with bleeding (BLE) and without bleeding (NON-BLE) on months 1 and 12 of frozen (-20ºC) storage.

In contrast, Viji et al. [22] reported a decrease on chewiness in bled and un-bled samples during 30 days of refrigerated storage. Jing et al. [42] documented a decrease in chewiness in bled and un-bled tilapia (*Oreochromis* sp.) throughout 12 days of iced storage. Addeen et al. [38] observed similar chewiness in chicken patties from bled and un-bled animals.

To the best of our knowledge, this is the first study evaluating the bleeding influence on the color and texture of *A. gigas* under frozen storage. Therefore, other meat matrices were used for comparison purposes in the discussion, highlighting the novelty and scientific importance of the present study.

Principal Component Analysis (PCA)

The PCA explained 90.61% of the total data variance (Figure 6). The first principal component (PC1) contributed to 63.21% of this variance and separated month 1 (M1) from 12 (M12) independent of bleeding (BLE x NON-BLE). Muscle pH, WHC, lightness (*L** values), yellowness (*b** values), hardness, and springiness presented square cosines greater than 0.6 and were relevant to this separation. At the beginning of storage (month 1; M1), NON-BLE samples exhibited greater values for pH, redness (*a** values), R630/580, and springiness, whereas lower values for lightness (*L** values) and yellowness (*b** values) for NON-BLE samples at month 12 (M12).

The second principal component (PC2) contributed to 27.40% of the variance. Despite the lower percentage, this component separated the samples of BLE M12 based on the square cosine of cohesiveness. Additionally, the combination of PC1 and PC2 resulted in the separation of the 4 groups: NON-BLE M1, BLE M1, BLE M12 and NON-BLE M12.

Conclusions

The findings of the present study indicate that bleeding influenced the quality attributes of pirarucu loins. BLE exhibited higher lightness and lower hardness and chewiness than their NON-BLE counterparts. Therefore, bleeding contributes to the quality of frozen pirarucu valuing the pirarucu chain and with a positive effect on local fisheries management.

Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, for providing the master scholarship to Fernanda Nascimento Teixeira. 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 (IDSM). We thank the Raízes do Purus Project, carried out by OPAN with sponsorship from Petrobras, for their support in collecting data for the research. 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).

References

1. Valladão GMR, Gallani SU, Pilarski F. South American fish for continental aquaculture. Rev Aquacult. 2018; 10: 351-369.
2. Cortegano CAA, Godoy LC, Petenuci ME, et al. Nutritional and lipid profiles of the dorsal and ventral muscles of wild pirarucu. Pesqui Agropecu Bras. 2017; 52: 271-276.
3. IBGE. Sistema IBGE de Recuperação Automática-SIDRA: tabela 3940: produção da aquicultura, por tipo de produto-ano 2020. 2022.
4. Constantino PAL, Silvius K, Alvarenga F, et al. Fishes and cowboy boots: An optimistic view. Conserv Sci Pract. 2020; 2: e252.
5. Campos-Silva J, Peres C. Community-based management induces rapid recovery of a high-value tropical freshwater fishery. Sci Rep. 2016; 6: 34745.
6. Fogaça FHS, De Oliveira EG, Carvalho SEQ, et al. Yield and composition of pirarucu fillet in different weight classes. Acta Sci Anim Sci. 2011; 33: 95-99.
7. Bassil PE, Martins MX, Sandes PH, et al. Bleeding influencing color, physical-chemical quality, and texture profile of pirarucu (Arapaima gigas) from Amazon, Brazil. J Food Stud. 2002; 11: 1-20.
8. Erikson U, Sigholt T, Rustad T. et al. Contribution of bleeding to total handling stress during slaughter of Atlantic salmon. Aquac Int. 1999; 7:101-115.
9. Olsen SH, Joensen S, Tobiassen T. et al. Quality consequences of bleeding fish after capture. Fish Res. 2014; 153: 103-107.
10. AOAC. Official methods of analysis. (18 ed). Gaithersburg: AOAC international. 2012.
11. Quéguiner C, Dumay E, Cavalier C, et al. Reduction of Streptococcus thermophilus in a whey protein isolate by low moisture extrusion cooking without loss of functional properties. Int J Food Sci Technol. 1989; 24: 601-612.
12. Verbeken D, Neirinck N, Meeren PVD, et al. Influence of k-carrageenan on the thermal gelation of salt-soluble meat proteins. Meat Sci. 2005; 70: 161-166.
13. AMSA. Meat Color Measurement Guidelines (2 ed). Champaign: American Meat Science Association. 2012.
14. Huidobro FR, Miguel E, Blázquez B, et al. A comparison between two methods (Warner–Bratzler and texture profile analysis) for testing either raw meat or cooked meat. Meat Sci. 2006; 69: 527-536.
15. Erikson U, Misimi E, Fismen B. Bleeding of anaesthetized and exhausted Atlantic salmon: body cavity inspection and residual blood in pre rigor and smoked fillets as determined by various analytical methods. Aquac Res. 2010; 41: 496-510.
16. Alvarado CZ, Richards MP, O'Keefe SF, et al. The effect of blood removal on oxidation and shelf life of broiler breast meat. Poult Sci. 2007; 86: 156-161.
17. Digre H, Erikson U, Misimi E, et al. Bleeding of farmed atlantic cod: residual blood, color, and quality attributes of pre- and postrigor fillets as affected by perimortem stress and different bleeding methods. J Aquat Food Prod Technol. 2011; 20: 391-411.
18. Erikson U, Tveit GM, Bondø M, et al. On-board live storage of Atlantic Cod (Gadus morhua): Effects of capture stress, recovery, delayed processing, and frozen storage on fillet color characteristics. J Aquat Food Prod Technol. 2019; 28: 1076-1091.
19. Erikson U, Misimi E. Atlantic Salmon skin and fillet color changes effected by perimortem handling stress, rigor mortis, and ice storage. J Food Sci. 2008; 73: C50-C59.
20. Inohara K, Kimura I, Yuan C. Suppressive effect of ATP on autoxidation of tuna oxymyoglobin to metmyoglobin. Fish Sci. 2013; 79: 503-511.
21. Nakazawa N, Wada R, Fukushima H, et al. Effect of long-term storage, ultra-low temperature, and freshness on the quality characteristics of frozen tuna meat. Int J Refrig. 2020; 112: 270-280.
22. Viji P, Tanuja S, Ninan G, et al. Biochemical, textural, microbiological and sensory attributes of gutted and ungutted sutchi catfish (Pangasianodon hypophthalmus) stored in ice. J Food Sci Technol. 2015; 52: 3312-3321.
23. Sohn JH, Ushio H, Ishida N, et al. Effect of bleeding treatment and perfusion of yellowtail on lipid oxidation in post-mortem muscle. Food Chem. 2007; 104: 962-970.
24. Huff-Lonergan E, Lonergan SM. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 2005; 71: 194-204.
25. Nguyen MV, Phan LMT. Influences of bleeding conditions on the quality and lipid degradation of cobia (Rachycentron canadum) fillets during frozen storage. Turkish J Fish Aquat Sci. 2018; 18: 289-300.
26. Liu Z, Xiong YL, Chen J. Protein oxidation enhances hydration but suppresses water-holding capacity in porcine longissimus muscle. J Agricult Food Chem. 2010; 58: 10697-10704.
27. Mc Clements DJ, Decker EA. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J Food Sci. 2000; 65: 1270-1282.
28. Ramos EM, Gomide LAM, Fontes PR, et al. Meat color evaluation and pigment levels in bullfrog (Rana catesbeiana) slaughtered by different methods. Aquac. 2005; 245: 175-182.
29. Sterniša M, Dvořak P, Lund R, et al. Bleeding of common carp (Cyprinus carpio) improves sensory quality of fillets and slows oxidative and microbiological changes during refrigerated aerobic storage. Food Technol biotechnol. 2018; 56: 524-532.
30. Huang H, Wang, L, Xiong G, et al. Influence of bleeding

on myoglobin and meat quality changes of Channel catfish muscle during freeze-thaw cycles. J Food Process Preserv. 2021; 45: e15877.

31. Hughes J, Clarke F, Purslow P, et al. High pH in beef longissimus thoracis reduces muscle fiber transverse shrinkage and light scattering which contributes to the dark colour. Int Food Res J. 2017; 101: 228-238.
32. Ramanathan R, Mancini RA. Role of mitochondria in beef color: A review. Meat Musc Biol. 2018; 2: 309-320.
33. Richards MP, Hultin HO. Contributions of blood and blood components to lipid oxidation in fish muscle. J Agric Food Chem. 2002; 50: 555-564.
34. Jagadeeswaran P, Liu YC. A hemophilia model in zebrafish: analysis of hemostasis. Blood Cells Mol Dis. 1997; 23: 52-57.
35. Terayama M, Yamanaka H. Effects of bleeding on the quality of skipjack. Nippon Suisan Gakk. 2000; 66: 852-858.
36. Wang H, Luo Y, Ertbjerg P. Myofibrillar protein gel properties are influenced by oxygen concentration in modified atmosphere packaged minced beef. Food chem. 2017; 230: 475-481.
37. Rotabakk BT, Bleie H, Stien LH, et al. Effect of blood removal protocol and superchilling on quality parameters of prerigor filleted farmed Atlantic cod (Gadus morhua). J Food Sci. 2013; 79: E881-E886.
38. Addeen A, Benjakul S, Prodpran T. Slaughtering method affects lipid oxidation, microbial quality, textural properties and volatile profile of chicken patties during storage. Emirates Journal of Food and Agriculture. 2017; 29: 387-395.
39. Benjakul S, Bauer F. Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze–thaw cycles. J Sci Food Agric. 2000; 80: 1143-1150.
40. Sørensen JS, Bøknæs N, Mejlholm O, et al. Short-term capture-based aquaculture of Atlantic cod (Gadus morhua L.) generates good physicochemical properties and high sensory quality during frozen storage. Innov Food Sci Emerg Technol. 2020; 65: 102434.
41. Ruiz-Ramírez J, Arnau J, Serra X, et al. Relationship between water content, NaCl content, pH and texture parameters in dry-cured muscles. Meat Science. 2015; 70: 579-587.
42. Jing F, Hui H, Laihao L, et al. Effect of different slaughter methods on quality of Oreochromis sp. fillets. South China Fisheries Science. 2013; 9: 13-18.