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Effect of high hydrostatic pressure on the meat of collared peccaries (Tayassu tajacu) with different ages
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Full Length Research Paper

Effect of high hydrostatic pressure on the meat of collared peccaries (Tayassu tajacu) with different ages

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This study aimed to assess the effect of high hydrostatic pressure (HHP) applied in the post-rigor period, on the physical and chemical parameters of the meat (Longissimus thoracis et lumborum) of peccary (Tayassu tajacu) obtained from animals of different ages. Pressures ranged from 100 to 400 MPa were applied to the muscle of young (19 months) and adult (38 months) animals for the time required to reach the set pressure followed by immediate depressurization. In adult animals, the shear force decreased as pressure increased, with an increase in meat tenderness at pressures above 200 MPa (P ≤ 0.05). The electrophoresis results showed changes in the protein profiles of animals of both ages subjected to the different pressure levels, with different degrees of protein denaturation. The results suggest that high hydrostatic pressure was effective in tenderizing the meat of adult peccaries and influenced its color, with potential positive effects on meat quality. Future studies should further explore how consumers perceive these aspects.

Key words: Peccary, color, texture, cooking loss, high hydrostatic pressure (HHP).

INTRODUCTION

Demand for exotic biological sources of protein is rising globally due to the steady population growth, possibility of rising incomes, and urbanization. Increasing the consumption of variety meats is a possible solution that could provide consumers with affordable meat products as well as generate revenue and reduce waste in meat processing (Warren et al., 2020).

Meat quality attributes are directly related to the food experience of consumers, the characteristics due to processing, and shelf life. Relevant attributes include safety, nutritional value, flavor, color, and oxidative stability (Fruet et al., 2018). The importance of bushmeat as source of food for forest peoples calls for an appropriate benefit/risk
analysis. Although there is few data on the nutritional content of most common bushmeat species, the available studies demonstrate that bushmeat is an important source of fats, proteins, micro and macro-nutrients (Vliet et al., 2017).

Knowledge on the nutritional and sensory characteristics of wild meat provides a better basis for its use as food, ultimately expanding studies in gastronomy areas and stimulating the development of new products (Moraes et al., 2022).

The consumption of domestic or wild animal meat (bushmeat) is related to social and cultural factors, and bush meat consumption has assumed different meanings throughout history. The aspects that guide the consumption or not of certain species are usually based on beliefs and traditional taboos, although emotional factors may also be involved (e.g., pets) (Cawthorn and Hoffman, 2016). Meat quality is closely related to sensory characteristics, and, from this perspective, it is essential to highlight the greatly appreciated consumption of wild meats, especially in European countries. Regional dishes prepared with deer (Capreolus capreolus), chamois (Rupicapra rupicapra), and wild boar (Sus scrofa) can be found in most restaurants and local fairs in the Alps, Apennines, Central Europe, and Mediterranean (Demartini et al., 2018).

In this scenario, illegal hunting has been discouraged in favor of animal farming, contributing to the knowledge to the potential production of native wild species (Hoffman and Cawthorn, 2012) and promoting sanitary care. Some native wild animals with zootechnical potential for legal captive breeding have been studied for decades regarding their reproduction, behavior, health, and nutrition, and are considered species under domestication, such as the collared peccary (Pecari tajacu) (Albuquerque et al., 2016). In rural communities of the Brazilian Amazon, these animals are often hunted for consumption and illegal trade, representing a negative impact on the ecosystem (Chaves et al., 2019; El Bizri et al., 2019). However, bush meat consumption contributes to the food security of rural communities, not only with protein intake, but also as a source of essential micronutrients for maintaining health (Sarti et al., 2015). Collared peccary, an animal also known as wild pig or cateto, is found in areas that range from the semi-arid regions to tropical forests in Brazil and other countries in the Americas. Its meat is an excellent source of animal protein similar to cattle, while its hide is highly valued in the international market (Santos et al., 2009), justifying the growing efforts to increase its use in captive breeding programs (Mayor et al., 2007). Also, the quality of this meat product contributes to consumer health in several aspects, such as blood glucose control, strengthening of the immune system, and improved vitamins and healthy fatty acids intake (Albuquerque et al., 2016). The consumer demand for high-quality meat processed with minimum negative impact has led to a greater investigation and the adaptation of new food processing technologies for the meat industry. Among these technologies, high hydrostatic pressure processing (HHP) stands out. It can modify the texture of these food products, favoring meat tenderization depending on the processing conditions (Bajovic et al., 2012).

The commercial success of high hydrostatic pressure can be mainly attributed to the production of foods with similar sensory characteristics to the fresh product and a nutritional value usually superior to thermally treated foods. These aspects observed in foods processed by high hydrostatic pressure successfully fulfill the consumer demand for fresh foods and the food industry requirements for shelf-life extension. Furthermore, compared to conventional thermal processes, the lower energy demand and environmental benefits can also be considered when adopting this technology (Tsevdou et al., 2019).

High hydrostatic pressure (HHP) is a promising non-thermal processing technology, which has been widely used in food processing, conservation and has become a commercial technology (Li et al., 2021).

As a non-thermal processing technology, high hydrostatic pressure can be used for food modification without affecting the quality and flavour constituents. The effect of this technology on food is closely related to the treatment time (Zhang et al., 2022).

High hydrostatic pressure is widely applied to food processing industry and to modulate protein structure, as well as to improve its functional properties. With volume compression under HHP, the aggregation, denaturation and emulsifying properties of proteins might be affected due to the distant change between atoms, the breakage and formation of different chemical bonds, and the interplay among protein molecules (Bonfim et al., 2019). Overall, HHP could significantly change the secondary structure of protein including the decrease of α-helix relative content and the increase of β-fold content (Ding et al., 2022).

Few studies employing HHP have been conducted with bush meat. Therefore, this study aimed to evaluate the effects of high hydrostatic pressure on the physicochemical parameters of peccary meat (Tayassu tajacu).

MATERIALS AND METHODS

Four peccaries (castrated males) with 19 months of age and four with 38 months of age and raised in captivity were slaughtered at the headquarters of Embrapa Eastern Amazon (Embrapa Amazônia Oriental, Belém - PA) following humane slaughter methods (The study was presented and approved by the Research Ethics Committee of the Federal University of Rio de Janeiro (Plataforma Brasil CAAE: 37387014.0.0000.5257)). The primary cuts (rib and loin) were packed in properly identified plastic polyethylene bags, placed under refrigeration, and immediately shipped by air to Embrapa Food Technology (Embrapa Agroindústria de Alimentos) in Rio de Janeiro - RJ wrapped in
secondary thermal packaging and under layers of ice to maintain the temperature close to 12°C. Upon arrival, the cuts were deboned in the post-rigor period. For this experiment, the muscle *Longissimus thoracis et lumborum* was cut into 2.5-cm-thick steaks, vacuum packed (Selovac 200B at vacuum level 20 or 50 Pa), and stored at 5°C until the pressurization process. Three samples were used per treatment, per replication, with six replicates for each treatment.

**High hydrostatic pressure (HHP)**

The muscle samples of *L. thoracis et lumborum* from animals with 19 and 38 months were pressurized using laboratory equipment (Stansted Fluid Power, model S-FL-850-9-W, England) with the capability to operate at a nominal pressure range from 100 to 900 MPa, under temperatures from 0 to 80°C, and different time intervals. The meat cuts previously stored in plastic bags, as mentioned previously, were placed in a stainless-steel cylinder (pressure vessel) with a total volume of 377 ml and a usable volume of 250 ml. The cylinder has orifices in its wall through which the pressurizing fluid circulates, in this case, 70% ethanol. Four pressure levels were used (100, 200, 300 and 400 MPa) with time named as zero, meaning that depressurization was carried out immediately after achieving the desired pressure (T0: Pressurization time required to reach the desired pressure). After inserting the samples in the cylinder, the pressurization chamber was hermetically sealed before beginning the operation using a system of mechanical seals to prevent leakage. First, a pneumatic pump was activated and injected a pre-load until the seals were closed. Subsequently, a second hydraulic pump automatically activated a piston that increased the pressure until the desired working condition, thus characterizing two pressurization stages. The equipment operated at a pressurization rate of 7 MPa/s, until reaching the desired pressure. The inlet temperature of the meat was established at 5°C to counterbalance any slight temperature increase due to adiabatic heating. At the end of the cycle, the chamber was opened after depressurization, and the pressurized samples were removed from the cylinder and stored at -18°C until the respective analyses. Figure 1 shows the processing stages until the analyses of peccary meat.

![Flow chart of peccary meat processing by high hydrostatic pressure. *Immediate depressurization after reaching the set pressure level.* Source: Authors.](image)

**Physical and chemical analyses**

The analyses described subsequently were performed with muscles from animals slaughtered at two ages (19 and 38 months), at four pressure levels (100, 200, 300 and 400 MPa) and without pressurization (0 MPa) as a control that remained under atmospheric pressure, and stored at -18°C, totaling 10 samples for each parameter evaluated. The samples were thawed in a fridge at 6 ± 2°C, 24 h before each analysis.

**Moisture, protein, lipids, and fixed mineral residue determination**

The analyses of moisture, protein, lipid, and fixed mineral residue (ash) followed the methodologies described in AOAC (1995) and were performed with raw muscles to characterize the meat samples.

**Cooking loss and shear force determination**

Ten steaks from each sample (approximately 2.5 cm thick) were weighed on an analytical balance, wrapped in aluminum foil, and roasted in an electric oven (Mondial fast grill, model S-12) with heating elements on both surfaces at 180°C until the internal temperature reached 72 to 75°C, controlled using a metal probe thermometer inserted into the central region of the steak.

To determine the cooking loss, the samples were cooled at
ambient temperature for one hour, surface moisture was removed with absorbing paper, and the samples were weighed again. The difference between the initial weight (before cooking) and the weight after cooking and cooling corresponds to the cooking loss. The values were expressed as percentages.

Sample preparation for shear force analysis followed the same procedure used for cooking loss determination. After cooking at ambient temperature, the samples were weighed, packed in previously identified plastic bags, and kept under refrigeration for 24 h (AMSA, 1995) at 5°C until the following day. Six cylindrical samples measuring 1.25 cm in diameter were cut from each steak longitudinally to the muscle fibers using an appropriate tool and used for shear force determination using a TA-Hdi texture analyzer (Texture Technologies Corp./ Stable Micro Systems, UK) equipped with a Warner-Bratzler shear blade (1 mm thick). The equipment was calibrated with a 50 kg weight. The crosshead speed was adjusted at 200 mm/min (AMSA, 1995), and the distance from the platform was 25.0 mm. Each cylindrical sample was cut only once, and the result was expressed in N (Newton).

**Instrumental color analysis**

The color of the raw muscle was analyzed by the CIE L*a*b system using a Hunter Lab colorimeter, model Color Quets XE, calibrated with a white standard with a 1 cm aperture. Six replications were used for each sample.

In the present study, the instrumental analysis of the meat color of the nine samples (unpressurized and eight HPP samples) was performed in the raw meat by reflectance using the Color Quest XE by Hunter Lab, scale CIE L*a*b and CIELCh instrument with an opening of 25 mm in diameter, illuminant D65, and observer 10. L* is the luminance or lightness component, which ranges from 0 (black) to 100 (white). Parameter a* varies from green to red (-80 to 0 = green; 0 to +100 = red), and b* shows the intensity from blue to yellow (-100 to 0 = blue, from 0 to +70 = yellow) (Papadakis et al., 2000).

**Protein profile**

Protein extraction was performed using 10 g from each raw sample, which was homogenized in 30 mL of extracting solution (0.065 M of Tris-HCl, pH 6.8, 3% sodium dodecyl sulfate (SDS) and 1% β-mercaptoethanol (ME)), allowing the concomitant extraction of sarcoplasmic and myofibrillar proteins. After homogenization for two minutes, the samples were centrifuged for 15 min at 4°C and 3,000 rpm (Bradford, 1976).

A 3 mL aliquot from the supernatant obtained after centrifugation was used for protein electrophoresis in polyacrylamide gel using a vertical electrophoresis system PROTEAN II xi Cell by BIORAD (Laemmli et al., 1970). Acrylamide was used at a concentration of 12% in the resolving gel and 4% in the stacking gel. The electrophoretic run was performed for seven hours at a voltage of 100V. The gel proteins were stained overnight with 10% acetic acid (v/v), 40% methyl alcohol (v/v), and 1% Coomassie Brilliant Blue R250 (v/v). The gel was destained in a solution containing 10% acetic acid (v/v) and 40% methyl alcohol (v/v) by renewing the solution every 30 min until the background was clear. The molecular weight of the protein fractions was calculated using standard curves with the molecular weight of the markers plotted against the respective distances in the gel.

The high molecular weight markers (Bio-Rad Laboratories, Richmond, USA) were myosin (202.44 kDa), β-galactosidase (116,58 kDa), bovine serum albumin (98.08 kDa), and ovalbumin (49.49 kDa), while the low molecular weight markers were phosphorylase B (103.04kDa), bovine serum albumin (80.66 kDa), ovalbumin (49.49 kDa), carbonic anhydrase (36.55 kDa), soybean trypsin inhibitor (28.83 kDa) and lysozyme (19.45 kDa).

**Fatty acid profile**

The fatty acid profile analysis used the methods of lipid extraction (Bligh and Dyer, 1959), esterification (Hartman and Lago, 1986), and gas chromatography in raw samples. The gas chromatograph used (Shimadzu GC-17 A) was equipped with a split/splitless injector (split ratio: 75:1), flame ionization detector, and an SP-2560 SUPELCO fused-silica capillary column (100, 0.25 and 0.2 μm). Chromatographic analysis was performed at an initial temperature of 170°C, increasing by 2°C/min until 215°C, 0.5 °C/min until 225°C, and 1°C/min until 240°C, being maintained at this temperature for 10 min. The injector and detector temperatures were 250 and 270°C, respectively. The gas flow rates used for the column were 0.49 mL/minute and a total flow rate of 61 mL/minute. The peaks were identified and quantified by comparison with standards (189191A FAME MIX C4 - C24). A 1 μL volume of the sample was used for injection. Quantification was performed by normalization, and the area percentages were transformed into mass percentages.

**Statistical analysis**

The samples were analyzed in triplicate; ANOVA and Tukey test were performed, using the R software.

**RESULTS AND DISCUSSION**

**Centesimal composition**

The centesimal composition results of the control (0 MPa) and pressurized peccary meat samples (100, 200, 300 and 400 MPa; immediate decompression after reaching the set pressure) from animals with different ages are shown in Table 1. High hydrostatic pressure had little effect on the centesimal composition of meats and meat products, and, as expected, there was little change in the moisture, ash, lipid and protein contents.

The moisture means showed little variation between the control (73.16 and 75.14%) and pressurized samples (varying from 74.77 to 77.39%) for both ages, although they differed significantly. According to ANVISA (2001), a maximum moisture value of 70% is required for unprocessed meat; however, this value refers to meats in general and is not specific for bush meat. According to Rodrigues and Andrade (2004), this higher moisture may be related to the lower fat content of peccary meat as water is mostly located in the muscle.

Moraes et al. (2022) studied the use of peccary meat as an alternative source of protein and found protein values (18.25%) lower than those found in this study, this can be explained by the fact that the author has prepared peccary sausage. Giménez et al. (2015) studying the physicochemical characteristics of unprocessed bovine meat in Adductor femoris and Semimembranosus muscles subjected to high hydrostatic pressure, reported 21.56, 1.42, 0.63 and 75.64% for protein, lipid, ash, and moisture, respectively.

According to Huang et al. (2020) high hydrostatic pressure affects non-covalent bonds such as hydrogen, hydrophobic, and ionic bonds, inducing changes in the
Table 1. Centesimal composition of control (0 MPa) and pressurized peccary meat from animals slaughtered at 19 and 28 months of age.

| Age (months) | P (MPa) | Moisture* (%) | Ash* (%) | Lipids* (%) | Protein* (%) |
|--------------|--------|---------------|----------|-------------|--------------|
| 19           | 0      | 75.14±1.680<sup>bc</sup> | 1.35±0.040 | 0.50±0.003 | 22.06±1.020<sup>b</sup> |
| 19           | 100<sup>f</sup> | 76.67±1.120<sup>ab</sup> | 1.33±0.020 | 0.61±0.002 | 22.0±1.060<sup>b</sup> |
| 19           | 200<sup>f</sup> | 77.39±1.920<sup>a</sup> | 1.34±0.030 | 0.49±0.005 | 22.3±1.020<sup>b</sup> |
| 19           | 300<sup>f</sup> | 76.10±1.230<sup>ab</sup> | 1.39±0.040 | 0.41±0.003 | 22.46±1.080<sup>b</sup> |
| 19           | 400<sup>f</sup> | 74.78±2.366<sup>cd</sup> | 1.37±0.060 | 0.34±0.005 | 24.81±1.040<sup>ab</sup> |
| 38           | 0      | 73.16±2.730<sup>d</sup> | 1.33±0.030 | 0.81±0.002 | 20.68±1.30<sup>c</sup> |
| 38           | 100<sup>f</sup> | 77.17±1.210<sup>a</sup> | 1.29±0.020 | 0.77±0.006 | 19.81±1.020<sup>c</sup> |
| 38           | 200<sup>f</sup> | 76.15±1.740<sup>ab</sup> | 1.22±0.010 | 0.76±0.005 | 22.62±1.050<sup>b</sup> |
| 38           | 300<sup>f</sup> | 74.77±1.840<sup>cd</sup> | 1.31±0.020 | 0.66±0.003 | 23.56±1.070<sup>ab</sup> |
| 38           | 400<sup>f</sup> | 75.20±1.13<sup>bc</sup> | 1.26±0.02  | 0.52±0.006 | 20.06±1.06<sup>c</sup> |

Means with the same letters in the same column do not differ (P > 0.05) by Tukey’s test. P = Pressure; <sup>f</sup>Immediate decompression after reaching the set pressure. <sup>a</sup>Mean of three replications.

Source: Authors

Table 2. Cooking loss, shear force, and tenderness variation in peccary meat (Longissimus thoracis et lumborum muscle) from animals of different ages subjected to different pressure levels.

| Age (months) | Pressure (MPa) | Cooking loss (%) | Tenderness variation (%) | Shear force (N) |
|--------------|---------------|------------------|--------------------------|-----------------|
| 19           | 0**           | 16.68 ± 0.75<sup>d</sup> | -                        | 15.29±0.73<sup>c</sup> |
| 19           | 100           | 21.59 ± 0.75<sup>cd</sup> | -7.40                    | 19.48±0.69<sup>a</sup> |
| 19           | 200           | 22.37 ± 2.26<sup>c</sup> | -15.95                   | 17.73±1.74<sup>b</sup> |
| 19           | 300           | 23.14 ± 0.89<sup>c</sup> | -14.58                   | 17.52±1.23<sup>b</sup> |
| 19           | 400           | 20.98 ± 2.79<sup>d</sup> | -19.55                   | 18.28±0.77<sup>b</sup> |
| 38           | 0**           | 22.92 ± 2.26<sup>c</sup> | -                        | 19.85±1.21<sup>a</sup> |
| 38           | 100           | 29.23 ± 4.43<sup>a</sup> | 3.73                     | 19.11±1.64<sup>b</sup> |
| 38           | 200           | 27.63 ± 3.13<sup>b</sup> | 9.87                     | 17.89±0.95<sup>b</sup> |
| 38           | 300           | 27.85 ± 3.76<sup>b</sup> | 12.69                    | 17.33±0.87<sup>b</sup> |
| 38           | 400           | 21.69 ± 2.75<sup>c</sup> | 10.22                    | 17.82±0.58<sup>b</sup> |

<sup>a</sup>Sample means with letters in common in the same column do not differ at 5% significance (P > 0.05) by Tukey’s test. <sup>b</sup>Control sample.

Source: Authors

Physicochemical properties and functional activities of macromolecules in food products and even resulting in protein denaturation. The effect of high hydrostatic pressure on proteins is dependent on the structure of the macromolecule and the composition of the medium (pH, ionic strength and temperature).

Myers et al. (2013) analyzed the protein content of turkey meat (19.50%), which was lower than in the present study; however, the fat content (0.97%) was higher than in peccary meat, which is leaner than turkey meat. This characteristic may attract consumers that seek a lean animal protein processed by high hydrostatic pressure, thus allowing a potentially higher added value. Collared pecaries are herbivores that feed mostly on leaves, branches, and fruits, in addition to a low energy demand (148.5 kcal/kg/day) that may favor a lower lipid content in wild compared to domesticated animals. Jardim et al. (2003) corroborated these results when they reported that the mean lipid content in the LTL (L. thoracis et lumborum) muscle of capybaras was 0.65 g/100 g in males and 1.09 g/100 g in females. Moreover, no change in the lipid content was observed after pressurization from 200 to 500 MPa in pork (Longissimus thoracis et lumborum) as expected, with only a slight increase in the fatty acid content at pressures from 350 to 500 MPa (He et al., 2012).

Previous results on peccary meat reported by Silva et al. (2002) differed from the present about moisture (71.21%), proteins (19.57%), lipids (7.96%), and ash (0.81%). This difference may have been due to the type of breeding system as the animals were not raised in captivity but free in nature and without a balanced diet, influencing the centesimal composition of their meat.

Cooking loss and shear force

Table 2 shows the results of cooking loss (CL),
tenderness variation, and shear force in the peccary meat samples. CL corresponds to the water volume lost during cooking. The shear force represents the pressure during chewing and is an indication of meat tenderness; the lower this pressure, the more tender the meat. Tenderness variation for each animal age was calculated in relation to non-pressurized meat (control).

Regarding cooking loss, all pressurized samples of younger animals showed greater cooking loss than the control, whereas, for the animals slaughtered at 38 months, only those samples subjected to 400 MPa did not differ from the control, and the remaining pressure levels caused greater losses compared to the control. It is worth noting that elevated HHP levels are undesirable as they indicate water loss during cooking, resulting in tougher, less juicy meats. Cooking loss in pressurized samples is due to the protein denaturation caused by high hydrostatic pressure, causing the meat to lose more water during cooking.

McArdle et al. (2010) and Kim et al. (2007) reported significant losses in bovine meat subjected to pressures higher than 300 MPa, suggesting a negative effect of high pressure levels on the water binding properties of meat, which was attributed to myofibrillar changes related to the severe contraction caused by high pressure levels (Marcos et al., 2010). Souza et al. (2011) evaluated the effects of high pressure processing on pork and reported that the cooking loss at 200 MPa (17.01%) was lower than in the control sample (20.58%), with a decrease in cooking loss in the pressurized sample, unlike the observations of the present study, in which the control samples showed the lowest cooking losses. Okamoto and Suzuki (2002) evaluated the effects of high pressure (100 to 500 MPa) on thawed pork and found means ranging from 14.92 to 18.93%, below those of the present study. For this parameter, the pork meat control (non-pressurized) sample showed 15.31% in the cited study.

Study carried out by Janardhanan et al. (2022) were evaluated loin samples (biceps femoris) from steers submitted to high hydrostatic pressure, where a significant effect of HPP on texture was observed, a behavior similar to that analyzed in this study in relation to older peccary meat.

The pressurization of muscle samples from younger animals had no positive effect on tenderness. The sample subjected to 100 MPa had the highest shear force, indicating less tenderness among the pressurized samples; however, a decrease in shear force values was observed with the increase in pressure, although they were still higher than the value required for unprocessed samples (15.29N). The fact that the meat from younger animals is tenderer may have contributed to minimizing the effect of high pressure.

The shear force in samples from older animals decreased with the increase in pressure, suggesting that the meat samples became tenderer. The samples pressurized at 200, 300 and 400 MPa did not differ from each other ($P > 0.05$) and achieved higher tenderness than the control sample, indicating that HHP had a positive effect on this parameter, with a significant increase in meat tenderness (9.87 to 12.69%). The control sample and the sample pressurized at 100 MPa did not differ for this parameter.

In another study, the mean shear force in the meat of male and female adult pigs was 37.16N (Leal et al., 2014), higher than the values found for animals slaughtered at 19 and 38 months in the present study (15.29 and 19.85N, respectively), and also compared to the results reported by Figueiredo (2016), highlighting the suitable performance of peccary meat with regard to this parameter. Souza et al. (2011) studied the effects of high-pressure processing (215 MPa/ 15s) on pork (L. thoracis et lumborum) and observed a significant difference between the control (24N) and pressurized (19N) samples, attesting the effect of HHP on meat tenderization.

In a study carried out by Han et al. (2021), a significant decrease in shear force was observed in samples submitted to HPP when the pressure increased from 0.1 to 300 MPa, indicating that the HHP treatment improved the tenderness of beef jerky ($P < 0.05$). The reduction in SF value as a result of the high-pressure treatment may be caused in part by protein conformational changes and denaturation of the muscle proteins (Cap et al., 2020).

HHP has been mostly used in studies with bovines. Neto et al. (2011) reported that the high-pressure processing of bovine meat from 200 to 300 MPa increased the release of cathepsins, increased the activity of calpains (by increasing the release of Ca$^{2+}$ from the sarcoplasmic reticulum), and inhibited calpastatins, resulting in meat tenderization. Above 400 MPa, Ma and Ledward (2004) reported a decrease in tenderization, possibly suggesting the inactivation of the enzymes responsible for pressure-induced tenderization. Cathepsins are responsible for meat tenderization but are inhibited by calpastatins, which sequester calcium and prevent its availability for cathepsins. However, pressure processing inactivates the calpastatins and favors tenderization by cathepsins.

**Instrumental color analysis**

The means of the instrumental color parameters in the control and pressurized peccary meat samples slaughtered at different ages are shown in Table 3.

High-pressure processing increased the $L^*$ values (lightness) in the meat of animals with 19 months, except for the sample pressurized at 100 MPa, whose mean for this parameter did not differ from the control ($P > 0.05$). Considering the animals with 38 months, this increase was observed in the samples pressurized at 200 and 400 MPa. The increase in this parameter in red meat is generally unsuitable, as high lightness values indicate pale meats, and consumers value a more intense red
color when purchasing this type of meat. However, this result is positive for peccary meat as a paler meat is already expected for this product.

Cap et al. (2020) indicated that beef meat treated with high pressure (200-350 MPa) led to an increase in L* values, which may be caused by globin denaturation and/or oxidation of myoglobin. The colour of the meat is not only determined by pigment proteins but is also influenced by alterations in muscle microstructure (Han et al., 2021).

Marcos and Mullen (2014) reported a significant increase of L* in pressurized bovine meat compared to untreated samples, with the highest means for samples treated at 400 and 600 MPa. This increase can be explained by changes in sarcoplasmic and myofibrillar proteins, resulting in changes on the surface of the meat (Jung et al., 2003), myofibrillar disorganization, and increased light reflection (Campus et al., 2008; Grossi et al., 2012). The loss of redness in meat samples, observed by the decrease in the a* parameter, may occur due to globin denaturation and/or to heme displacement or release, and also due to the partial oxidation of ferrous myoglobin to ferric myoglobin. Globin denaturation facilitates the action of oxidizing agents in the medium, resulting in the oxidation of the ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions (Campus et al., 2008) and the appearance of a brownish color like cooked meat. Among the pressurized samples with 19 months, all differed significantly from the control with regard to the intensity of red (a*). For the pressurized samples with 38 months, only the sample subjected to 100 MPa differed from the others (P < 0.05). The samples with 38 months subjected to 100 and 200 MPa showed a loss of redness compared to the control due to myoglobin oxidation, with a consequent decrease of a* as the samples pressurized at 300 and 400 MPa did not differ significantly from the control.

Studies conducted by McArdle et al. (2010) with pressurized bovine meat observed significant differences between the control sample and those processed at 300 MPa/40°C. However, McArdle et al. (2013) reported that HHP did not affect this parameter (a*) in lamb meat processed at 400 and 600 MPa. Similar study focusing on goat meat processed at 100 MPa/10 and 20 min revealed lightness (L*) means below the present study (Cantoia and Feihmann, 2017), suggesting that peccary meat is paler than goat meat for both the control and pressurized samples. Color is a crucial quality attribute during purchase, and the results obtained contribute to consumer acceptance. Villamonte et al. (2013) analyzed the color parameters of pressurized cooked pork (0.1 and 350 MPa) and observed a decrease in the L* values (64.38 and 53.90, respectively) with the increase in pressure, in addition to increased values of a* (1.37 and 1.76, respectively), with similar behaviors as those verified in the present study.

### Protein profile

The polyacrylamide gel electrophoresis data (SDS-PAGE) confirmed some changes in the protein profiles when the meat samples from both younger (19 months) and older animals (38 months) were treated at different pressures. Differences were observed between the protein fractions as a function of the age at slaughter and high hydrostatic pressure.

Figure 2 shows the electrophoretic profile of the control (columns A and F) and pressurized samples (100 to 400 MPa: columns B - E and G - J), whose polypeptide chains mostly show molecular weight variations within the range of the standards used (16.76 to 127.4 kDa).

The electrophoretic profile identified the presence of 15 different protein bands corresponding to proteins and

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**Table 3.** Instrumental color parameters of unprocessed (control) and pressurized peccary meat from animals slaughtered at 19 and 28 months of age.

| Age (Months) | Pressure (MPa) | L*     | a*     | b*     |
|--------------|---------------|--------|--------|--------|
| 19           | 0**           | 46.84±1.28<sup>a</sup> | -1.37±0.4<sup>e</sup> | 6.21±1.84<sup>bc</sup> |
| 19           | 100           | 46.32±1.94<sup>d</sup> | -0.76±0.08<sup>d</sup> | 6.47±2.94<sup>de</sup> |
| 19           | 200           | 52.83±2.31<sup>b</sup> | -0.94±0.2<sup>d</sup> | 8.29±1.78<sup>bc</sup> |
| 19           | 300           | 52.51±1.48<sup>b</sup> | -3.09±0.8<sup>f</sup> | 5.83±1.71<sup>a</sup> |
| 19           | 400           | 51.80±2.95<sup>bc</sup> | -0.29±0.05<sup>cd</sup> | 8.40±2.16<sup>b</sup> |
| 38           | 0**           | 48.06±1.29<sup>d</sup> | 1.73±0.08<sup>a</sup> | 9.52±1.96<sup>b</sup> |
| 38           | 100           | 49.25±1.58<sup>cd</sup> | -1.27±0.3<sup>de</sup> | 7.27±1.53<sup>c</sup> |
| 38           | 200           | 52.22±2.83<sup>b</sup> | 0.95±0.02<sup>bc</sup> | 9.94±1.23<sup>b</sup> |
| 38           | 300           | 48.36±1.20<sup>d</sup> | 1.37±0.09<sup>ab</sup> | 8.95±1.43<sup>b</sup> |
| 38           | 400           | 63.66±2.62<sup>a</sup> | 2.82±0.14<sup>a</sup> | 13.32±1.97<sup>a</sup> |

<sup>a</sup>Means with the same letters in the same column do not differ (P < 0.05) by Tukey's test.  L = lightness (0 = black and 100 = white); a= intensity of green/red (-80 to zero = green, from zero to +100 = red), b= intensity of blue/yellow (-100 to zero = blue, from zero to +70 = yellow). Mean of six replications. **Control samples.

Source: Authors
myofibrillar protein fragments typical of muscles, e.g., actin, myosin, actinin, troponin, and tropomyosin. High hydrostatic pressure had an effect on the protein profile, as seen by the large amount of protein fragments, which were possibly conformationally altered by high pressure. Figure 2 shows the higher color intensity of the myosin (127.4 Kda) and actin bands (44.15 Kda), and probably of fragments of myosin light chain, with regard to troponin T or subunits of β-actin (37.5 Kda). These results are expected for the characterization of myofibrillar proteins and were similar to those reported by Daguer et al. (2010); however, the presence of heavy myosin chains was not observed (223 Kda). Also, other myofibrillar protein fractions were observed, such as α-actinin (101.36 kDa), troponin (52.9 kDa), and β-tropomyosin (33.98 kDa). As expected, the electrophoretic profiles of peccary meat showed low molecular weight bands corresponding to polypeptides resulting from protein degradation, as observed by Xiong et al. (2006) in pork. A faintly stained fragment of myoglobin (17 kDa) was observed among these bands. Peccary meat, as well as pork, has lower amounts of this pigment compared to bovine meat. Thus, the little conspicuousness of this band may be a reflection of the amount of myoglobin present in peccary muscles. However, the presence of bands with molecular weights up to 37 KDa may correspond to both myosin light chain fragments and troponin T, or even subunits of β-actin as the molecular weights of these proteins are similar and, therefore, their bands are confused in the electrophoretic profiles (Souza et al., 2004).

Xue et al. (2019) studied the effect of high hydrostatic pressure (100, 200 and 300 MPa) on proteins from meat products and reported that at 100 MPa, no visible changes in the protein profile was observed. However, when reaching pressures equal to or higher than 200 MPa, a marked decrease was observed in the intensities of some protein gel bands, especially those of high molecular weight. A similar behavior was observed in this study, especially in the peccary meat from older animals (38 months), in which changes in high molecular weight proteins were visible as pressure approached 400 MPa, such as in myosin (127.4 Kda). Moreover, the results suggested that the myofibrillar proteins were sensitive to high pressure, as observed in the study conducted by Chen et al. (2017).

Low pressure levels can affect tertiary and secondary structures or both protein structures in meat (Chen et al., 2017; Xue et al., 2019), interfering with their functionality, as reported by Xue et al. (2019).

**Fatty acid profile**

The fatty acids found at higher concentrations in the peccary meat from animals of both ages were palmitic (C16:1) and linoleic acid (C18:2 ω6). There was no statistical difference between the control and pressurized
samples ($P > 0.05$), suggesting that high hydrostatic pressure did not affect the fatty acid profile (Table 4).

The pectoral meat showed a higher content of unsaturated fatty acids (oleic, linoleic, linolenic, and eicosenoic) than saturated fatty acids (lauric, myristic, palmitic, and stearic) for both ages, regardless of the pressure levels. Albuquerque et al. (2010) observed concentrations of 3.90, 24.88, 13.51, 1.18 and 28.47g/100 g of myristic, palmitic, stearic, palmitoleic, and oleic acid, respectively in male peccaries (leg muscle) fed with babassu meal. These values, as well as the contents of saturated and monounsaturated fatty acids were higher than those reported by Fernandes et al. (2010); however, it should be noted that fat content in the legs is higher than in the loin.

Similar results were reported by Canto et al. (2015) for other meat products. The authors reported that different pressure levels (200, 300 and 400 MPa) did not affect the total saturated fatty acids in caiman meat. However, although the total fatty acids were similar in both meat samples, higher oleic acid means (monounsaturated) were observed in caiman meat, unlike the present study, in which linoleic acid (polyunsaturated) showed the highest concentration, highlighting the favorable nutrient content of peccary meat.

In goat meat, Ding et al. (2010) reported that pressures between 200 and 400 MPa did not affect the $\text{M. Pectoralis profundus}$ muscle regarding the contents of lauric, myristic, palmitoleic, and oleic acid. Similar results were found in the present study for the $\text{L. thoracis et lumbarum}$ muscle of peccaries of both ages. Kang et al. (2013) also reported that high-pressure processing did not affect the fatty acid profile of goat meat, with the vaccenic, palmitic and stearic acids showing the highest concentrations.

Table 4. Fatty acid profile in peccary meat from animals of different ages subjected to different pressure levels.

| Fatty acids (mg/100 g) | 19 months | 38 months |
|-----------------------|-----------|-----------|
|                       | Control   | 100 MPa   | 200 MPa   | 300 MPa   | 400 MPa   | Control   | 100 MPa   | 200 MPa   | 300 MPa   | 400 MPa   |
| C12:0 Lauric           | 1670 a    | 1170 a    | 1750 a    | 1450 a    | 1540 a    | 1530 a    | 1470 a    | 1780 a    | 1720 a    | 1490 a    |
| C14:0 Myristic         | 640 a     | 760 a     | 1000 a    | 320 a     | 630 a     | 940 a     | 830 a     | 800 a     | 910 a     | 870 a     |
| C16:0 Palmitic         | 1673 b    | 16670 b   | 16050 b   | 16490 b   | 16100 b   | 18570 b   | 18040 b   | 18350 a   | 18560 a   | 18650 a   |
| C16:1 Palmitoleic      | 630 a     | 510 a     | 620 a     | 350 a     | 540 a     | 860 a     | 830 a     | 760 a     | 720 a     | 790 a     |
| C18:0 Stearic          | 8360 ab   | 9010 a    | 9450 a    | 9730 a    | 9120 a    | 7790 b    | 7750 b    | 7520 b    | 7450 b    | 8010 ab   |
| C18:1 ω9 Oleic         | 8790 a    | 8800 a    | 8730 a    | 10150 a   | 8130 a    | 8050 a    | 8210 a    | 8670 a    | 8840 a    | 8460 a    |
| C18:2 ω6 Linoleic      | 25830 a   | 25560 a   | 26550 a   | 25880 a   | 25750 a   | 25680 a   | 25370 a   | 25750 a   | 25550 a   | 25420 a   |
| C18:3 ω3 Linolenic     | 360 a     | 270 a     | -         | 420 a     | 280 a     | 310 a     | 350 a     | 270 a     | 250 a     | 280 a     |
| C20:1 ω9 Eicosenoic    | 700 a     | 730 a     | 790 a     | 800 a     | 710 a     | 930 a     | 910 a     | 860 a     | 940 a     | 830 a     |
| Total SFA              | 27400 a   | 27610 a   | 28250 a   | 27990 a   | 27390 a   | 28830 a   | 28040 a   | 28450 a   | 28640 a   | 29020 a   |
| Total MUFA             | 10120 a   | 10040 a   | 10140 a   | 11300 a   | 9380 a    | 9840 a    | 9950 a    | 10290 a   | 10050 a   | 10080 a   |
| Total PUFA             | 26190 a   | 25830 a   | 26550 a   | 26300 a   | 26030 a   | 25990 a   | 25720 a   | 26020 a   | 25800 a   | 25700 a   |

*Means with different letters in the same row differ ($P < 0.05$) by Tukey's test.
Source: Authors

**Conclusion**

Pressurized samples of 38-month-old peccaries showed increasing on tenderness with the increasing pressure, suggesting a positive effect of HHP on this meat matrix. Regarding cooking loss, high-pressure processing showed that the best pressurization condition was 400 MPa, resulting in the lowest cooking loss, possibly due to the increase in the solubility of myofibrillar proteins.

Instrumental color analysis showed that HHP affected the $L^*$ parameter. The paler color of the muscle, expected for this meat product, can contribute to a better consumer perception at the time of purchase.

The electrophoretic profile showed that the meat proteins were affected by high pressure, especially those of low molecular weight, except actin, which showed greater integrity at different pressure levels.

There were no significant differences between the control and pressurized samples with regard to the fatty acid profile; however, the age of the animals played an important role in the means obtained. It is worth noting that peccary meat is rich in essential fatty acids such as linoleic acid, in addition to the presence of omega 3, 6 and 9 fatty acids. These characteristics are highly favorable and, if transmitted to the consumer, will be appreciated.

HHP improved the characteristics of peccary meat from adult animals, especially regarding tenderness, one of the most valued attributes by the consumer, and color,
given its effect on muscle lightness. However, the cost of this process might still hinder the adoption of this technology for conventional products, restricting it to “premium” high added value ones. That could be the case of bushmeat products, which should be evaluated in future consumer studies. Such product, with added value and extended shelf life, could favor the entire bush meat production chain, in addition to providing the consumer with an alternative meat option with appropriate nutritional characteristics.

Despite the demand for this product, collared peccary meat is scarce and not readily available for commercialization. In addition, further studies on the effects of high hydrostatic pressure on the characteristics of peccary meat are needed to add value to the product.

**CONFLICT OF INTEREST**

Authors declare no Conflict of Interests for this article.

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