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Use of ultrasound and acerola (*Malpighia emarginata*) residue extract tenderness and lipid oxidation of pork meat

Chimenes Darlan Leal de ARAÚJO[1], Gledson Firmino Gonçalves da SILVA[1], Jorge Luiz Santos de ALMEIDA[2], Neila Lidiany RIBEIRO[3]*, Leonardo Augusto Fonseca PASCOAL[1], Fábio Anderson Pereira da SILVA[1], Valquíria Cardoso da Silva FERREIRA[1], Terezinha Domiciano Dantas MARTINS[1]

Abstract

This work aims to evaluate the application of ultrasound and marinate with extract of acerola (*Malpighia emarginata*) residue on the tenderness and lipid oxidation of the meat pork. Samples of loin (*Longissimus dorsi*) containing approximately 90 g, 10 cm long, 5 cm wide, and 2 cm high were extracted from the pig carcass. The samples were coded and randomly allocated under a factorial scheme 2 (with and without antioxidant extract) x 3 (ultrasonic bath times 0, 5, and 10 minutes); each treatment had four replicates. The final extract volume was adjusted with distilled water, ensuring 200 mg of gallic acid equivalents per kilo of meat. Then, the samples were placed in the ultrasonic bath at a fixed frequency of 170 W, 35 kHz. During the ultrasound application, the bath temperature was maintained at 10 °C with ice/ice water addition when necessary. The acerola´s extract showed a significant effect ($P < 0.05$) on the meat Cohesiveness and to a lesser extent before rupture. With the modification of the microstructure in the meat, there was a decrease in elasticity. The application of ultrasound to meat (170 W, 35 kHz) in times (5 and 10 minutes) combined with the marinating in a natural antioxidant acerolas residue extract improves the meat's quality characteristics, decreasing the hardness and chewiness.

Keywords: ultrasound; meat quality; natural antioxidant; lipid oxidation; texture.

Practical Application: Acerola (*Malpighia emarginata*) is a fruit widely consumed globally and of great economic value in Brazil, recognized for being a good source of vitamin C, phenolic compounds, flavonoids, and anthocyanins, where they have an antioxidant potential. In combination with marinating, ultrasound causes muscle fibers' breakdown and provides better penetration of added fluids. In this context, the penetration between the fibers occurs more efficiently, leading to an improvement in the dispersion of the liquid in the meat.

1 Introduction

The tenderness of the meat is one of the most critical elements in the choice of the product. The change in the meat's intrinsic structure, such as increased proteolysis and fragmentation of the myofibrils, contributes to improving the tenderness (Xiong et al., 2020).

Some techniques promote changes in the physical structure of the meat, providing tenderization. Among the techniques, marinating by immersion and injection using equipment such are very highlighted in the literature. However, innovative techniques that are considered to be emerging are being investigated by researchers, such as ultrasonic waves and the combination of them with marinating technology (Alarcon-Rojo et al., 2019; Al-Hilphy et al., 2020).

The application of ultrasonic waves in meat generates cavitation formation caused by vibrational sound energy within the system, where small collapses occur in the intrinsic structure, contributing to protein degradation and fiber removal. Depending on the time and ultrasound intensity on the meat, rupture of myofibrils in the Z line, troponin, and myosin denaturation may occur, contributing to tenderization and improving the penetration of liquids in the marinating process (Yeung & Huang, 2017; Amiri et al., 2018; Wang et al., 2018; Alarcon-Rojo et al., 2019; Xiong et al., 2020). In combination with marinating, ultrasound causes muscle fibers' breakdown and provides better penetration of added fluids. In this context, the penetration between the fibers occurs more efficiently, leading to an improvement in the dispersion of the liquid in the meat (Alarcon-Rojo et al., 2019)

The liquids used for marinating commonly contain substances that cause the proteolytic action of the meat. However, in sum, this liquid can be a source of compounds that provide other benefits to the product's quality, such as the antioxidant action (Rezende et al., 2018). Antioxidants can be synthetic and natural. However, studies have been carried out to search for natural antioxidants' application since synthetic antioxidants may be associated with the triggering of chronic diseases to consumers

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[1]*Programa de Pós-graduação em Tecnologia Agroalimentar, Centro de Ciências Humanas, Sociais e Agrárias, Universidade Federal da Paraíba – UFPB, Bananeiras, PB, Brasil*
[2]*Programa de Doutorado Integrado em Zootecnia, Universidade Federal da Paraíba – UFPB, Areia, Paraiba, Brasil*
[3]*Instituto Nacional do Semiárido – INSA, Campina Grande, PB, Brasil*
*Corresponding author: neilalr@hotmail.com

(Lourenço et al., 2019). In this sense, several studies have embarked on the search for natural antioxidants from plants and fruit residues (Barbosa-Pereira et al., 2014; Guerra-Rivas et al., 2016; Reis et al., 2017; Chauhan et al., 2019; Domínguez et al., 2020).

Residues from fruits have antioxidant potential, being an alternative in reusing residues and substituting synthetic antioxidants (Rezende et al., 2018). Some of these discarded wastes are considered pollutants, but most can be used to feed animals, reducing production costs by turning low-nutrient residues into high-value products such as meat (Geron et al., 2015; Hassan et al., 2016). Among the fruits processed by agribusiness, acerola is widely used in the manufacture of beverages, syrup, ice cream, jams, jellies, caramel, juice, and dehydrated and canned products (Denny et al., 2013). Acerola (Malpighia emarginata) is a fruit widely consumed globally and of great economic value in Brazil, recognized for being a good source of vitamin C, phenolic compounds, flavonoids, and anthocyanins, where they have an antioxidant potential (Silva et al., 2019). The extraction of these compounds is essential for studies that prove their composition and antioxidant capacity. Therefore, isolation, quantification, identification of phytochemicals in plant sources of these compounds and evaluation of benefits that these present to health demonstrate the purpose of its use (Schafranski et al., 2019).

The Moura pig has a great commercial appeal, it is a Brazilian heritage that needs to be preserved. The excellence of the meat is compared to Iberian breeds such as the Alentejo and the Pata Negra, as they are all of the same genetic origin as the Moura (Favero et al., 2007). For ethical and economic reasons, a good experimental design requires the use of the minimum number of animals necessary to reach a desired objective given the required precision (Festing & Altman, 2002).

Therefore, this work's objective was to evaluate ultrasound application and marinate with acerola residue extract on the tenderness and lipid oxidation of pork meat in an isolated and combined way. And identify which characteristics have the best discriminatory power through principal component analysis.

2 Material and methods

2.1 The obtaining the acerola extract

The residue from the pulping of the acerola was obtained from the fruit pulp Agroindustry located in Bananeiras, in Paraíba, Brazil. The residue was transported in an isothermal box to the Physical-Chemical Analysis Laboratory (CCHSA-UFPB) and subjected to drying in an oven with air circulation at a temperature of 50 °C for 24 h. The dry residue was crushed in a Willey 41 knife mill (SL - 31, Solab, Piracicaba - SP, Brazil), sieved (10 mesh), packed in a vacuum polyethylene bag stored under deep freezing at -80 °C until the extraction.

Obtaining the antioxidant extract from the acerola residue was carried out according to Packer et al. (2015). Initially, the dry residue was mixed with 80% ethyl alcohol solution in the proportion of 1/10. The mixture was stirred manually for 5 minutes, standing for 10 minutes, being stirred a second time for 5 minutes. After this period, the solution was centrifuged for 15 minutes (centrifuge 320 R, Hettich, Tuttlingen, Germany) at 10 ° C and 320rpm. Then, the solution was filtered through qualitative filter paper, and the extract was subjected to evaporation in a vacuum evaporator (180 mbar at 45 °C) to remove ethyl alcohol from the solution. Finally, the extract was stored in an amber container and kept at -80 ° C in an ultra-freezer (MDF –U33V-PA, Panasonic Healthcare, Japan).

2.2 Experimental site and sample preparation

The Ethics Committee approved this research for the Use of Animals (CEUA) of the Federal University of Paraíba (protocol nº 8410081019).

The meat was obtained from four male pigs, castrated, from the genetic group (½Duroc x ½ Moura), aged 12 months (142 ± 4.3 kg). According to the Brazilian welfare codes of practice, all the pigs were slaughtered on the same day using standard commercial procedures (Brasil, 2000). The carcasses were chilled at 4 °C for 24 h in a cooling room. Samples of loin (*Longissimus dorsi*) containing approximately 90 g, 10 cm long, 5 cm wide, and 2 cm high were extracted from the swine carcass. Then, the samples were packed in polyethylene packages and stored at 4 °C to stabilize the temperature and then apply the natural antioxidant extract at different times of ultrasonic bath.

2.3 Application of antioxidant extract combined with ultrasound in meat

Acerola juice can be obtained in different ways, such as by pressing or extracting the pulp. Most of the residue from the processing of the acerola comes from the pressing steps to obtain the pulp and clarification to obtain the juice with a low solids content. After pressing, there is the residue called bagasse, characterized by the seed and bark (Albuquerque et al., 2019).

The final extract volume was adjusted with distilled water, ensuring 200 mg of gallic acid equivalents per kilo of meat. The extract was applied to the meat surface inside the package and homogenized. Then, the samples were placed in the ultrasonic bath (L220-SCHUSTER) at a fixed frequency of 170 W, 35 kHz. During the ultrasound application, the bath temperature was maintained at 10 °C with ice/ice water addition when necessary. After applying the extract and ultrasound, the samples were stored in a refrigerator (4 °C) for 24 hours.

2.4 Determination of texture profile

For the texture profile analysis, the CT3 Texture Analyzer (Brookfield) was used. For each experimental unit, four cooked samples were obtained (2 cm in length, 2 cm in width, and 1 cm in height, approximately), totaling 24 samples. The samples were subjected to cooking in a water bath until reaching an internal temperature of 72 °C. Two compression cycles of 50% deformation from the original height were used with a 38 mm probe at a speed of 2 mm/s. the parameters determined were according to Bourne (1982): hardness (N), adhesiveness (mJ), cohesiveness (dimensionless), elasticity (mm) and chewiness (N).

2.5 Determination of sarcoplasmic calcium content

To measure the sarcoplasmic calcium content, the methodology of Cheah et al. (1984) modified Cheah et al. (1986). Initially, 10 g of the sample were homogenized in 25 ml of KCl 150 mmol L^{-1}, and the mixture was centrifuged at 4000 rpm for 4 min at 5 °C (Universal 320 R.). The residue was discarded, and the supernatant was again centrifuged at 26000 g for 4 min at 5 °C. Then, 1.0 ml aliquots of the supernatant were transferred to test tubes, and 4.0 ml of 0.5% lanthanum solution was added. The reading was performed on an atomic absorption spectrophotometer, model iCE 3500 (Thermo Scientific, Cambridge, England), at 422.7 nm. For the standard, 2 ppm calcium carbonate was used.

2.6 Myofibrillary fragmentation index (MFI)

The determination of the MFI was carried out according to the methodology proposed by Olson et al. (1976) with modifications by Hopkins et al. (2000). The concentration of soluble proteins was determined by the Biuret method (Gornall et al., 1949) with a standard curve of 0 to 5 mg / mL. Sample aliquots were diluted with buffer solution to reach a final concentration of 0.5 mg / mL of proteins. With a spectrophotometer's aid at 540 nm, the readings' values were obtained and then multiplied by 200 to find the MFI.

2.7 Microstructure of meat

The samples (2 cm long, 2 cm wide, and approximately 1 cm high) were fixed in metacan solution (70% methanol, 20% chloroform, and 10% acetic acid) for 24 h to preserve the biological structure and then dehydrated in an air circulation oven for three hours. With the aid of a scanning electron microscope (TESCAN brand, model VEJA 3), the samples were deposited on a carbon ribbon on the shelf and sprayed with approximately 6 mm thick Au / Pd by sputtering (SPut Module Sputter Coater). The acceleration voltage varied from 5 to 15 kV.

2.8 Physicochemical analysis

The color was obtained through the Colorimeter (Minolta, Model CR-400, Japan), using the CIE system L*, a*, b*, determining the coordinates L* (luminosity), a* and b* (yellow index), illuminant C, observer angle 8° (Miltenburg et al., 1992). To evaluate the color, three readings were obtained in each animal's muscles, and the mean was calculated.

The measurement of meat pH was performed 24 h *post mortem* (Association of Official Analytical Chemists, 2005) using a digital potentiometer (DIGIMED, model pH 300M, São Paulo/Brazil), equipped with a glass electrode.

To determine cooking losses (CL) (evaporation, dripping, and totals; Wheeler, 1995), two samples (2 cm long, 2 cm wide, and approximately 1 cm high) were obtained, with the cut made in the muscle fibers of the *Longissimus lumborum* muscle. The steaks were thawed in a refrigerator overnight at 4 ° C, weighed on a precision scale (SHIMADZU, model TX3202L) and placed together in a roasting pan and roasting pan, and then roasted in an electric oven preheated to 150 °C (FISCHER, Star model), until the internal temperature of the samples reached the limit of 71 °C (monitoring obtained by type K thermocouples inserted in the geometric center of the sample; the reading was performed with a digital reader TENMARS, model TM-361). Subsequently, the samples were cooled to room temperature until the internal temperature reached 24 to 25 ° C through an insertion thermometer (TESTO, model 106). Afterward, the samples were weighed to obtain the samples' difference in weight before and after the cooking and expressed as a percentage.

2.9 Lipid oxidation

Lipid oxidation was determined by extracting substances that react to thiobarbituric acid (TBARS), quantified in a spectrophotometer following the method described by Ganhão et al. (2011). The absorbance reading was performed at 532 nm, using a standard curve of tetra ethoxy propane (TEP) to quantify the amount of malonaldehyde (MDA). The results were expressed in mg of MDA / kg of sample.

2.10 Statistical analysis

A completely randomized statistical design (DIC) was used, under a factorial scheme 2 (with and without antioxidant extract) x 3 (ultrasonic bath times 0, 5, and 10 minutes), each treatment with four repetitions. Analysis of variance (ANOVA) and data was applied when necessary, submitted to the Tukey comparison test with a significance level ($P < 0.05$) using the SAS University software (SAS Institute Inc, 2012).

After standardization, a multivariate analysis test was carried following the recommendations previously established by Sneath & Sokal (1975) to allocate the animals into groups according to similarity and verify the original traits' discriminatory power. The principal component analysis (PCA) allowed the overall variance assessment; it was performed by the PRINCOMP procedure, separately for each population. Besides, discriminant analysis was performed to describe the variation among groups, identifying those traits with the best discriminatory power.

3 Results

3.1 Texture profile

Cohesiveness showed a significant effect ($p < 0.05$) for the extract, the pork with the extract showed a lesser extent before rupture, and the elasticity had a significant effect ($p > 0.05$) for ultrasound, with the modification of the microstructure in the meat there was a decrease in elasticity (Figure 1).

Capital letters represent the effect ($p < 0.05$) of Extr (Natural antioxidant extract). Lower case letters represent the effect ($p < 0.05$) of Us (Ultrasound).

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The hardness and chewiness parameters had similar behaviors, where the presence of the extract and precisely in the time of 10 minutes of ultrasound ($p < 0.05$) obtained the lowest results.

3.2 Sarcoplasmic calcium, myofibrillar fragmentation index (MFI), and pH

The parameters of sarcoplasmic calcium and MFI showed a significant effect ($p < 0.05$) for the application of ultrasound (Figure 2). The incorporation of the natural extract had no significant effect on the parameters of MFI and sarcoplasmic calcium. However, pork exposure to ultrasonic waves under the conditions tested increased the release of sarcoplasmic calcium and promoted more significant fragmentation of myofibrils regardless of the application time. However, the pH did not change due to the application of ultrasound and extract.

Capital letters represent the effect ($p < 0.05$) of Extr (Natural antioxidant extract). Lower case letters represent the effect ($p < 0.05$) of Us (Ultrasound).

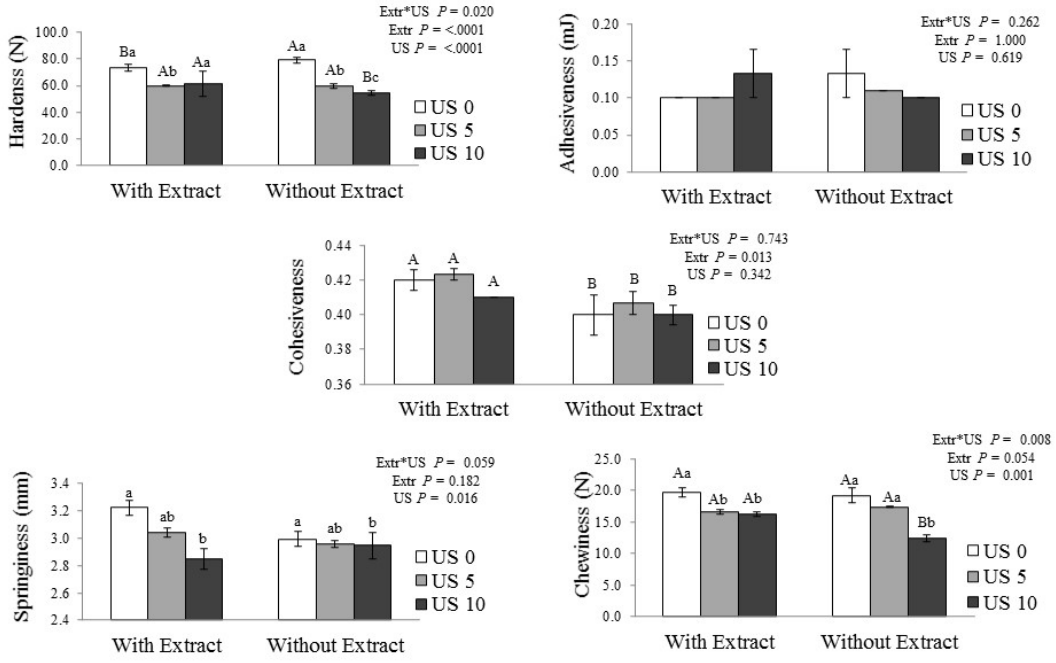

Figure 1. Effect of ultrasound application and natural antioxidant extract of acerola on the texture profile of pork. * Different uppercase letters between treatments with and without extract differ between each other and lowercase letters differ within the treatment by Tukey's test

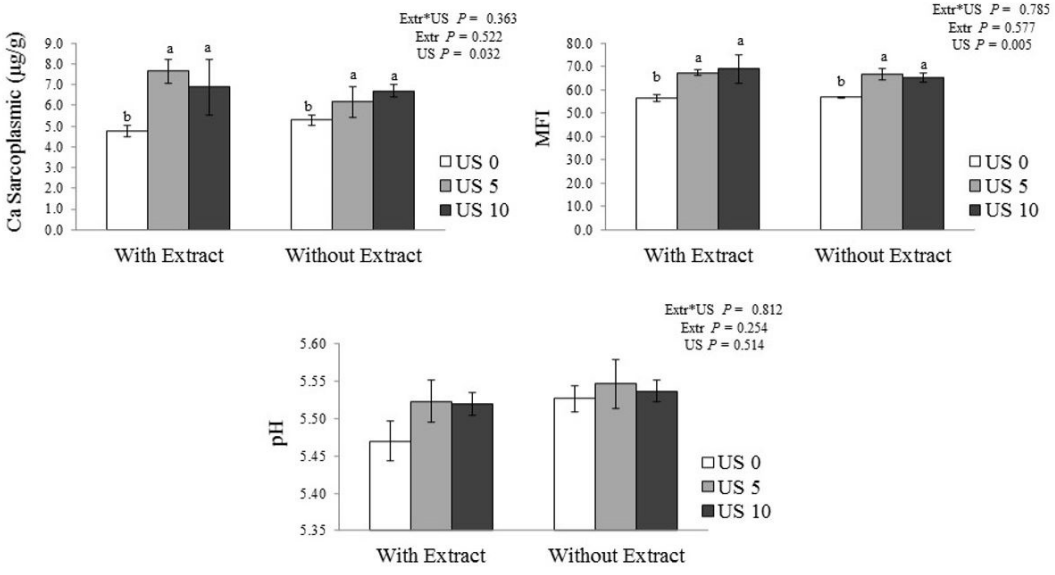

Figure 2. Effect of the application of ultrasound and natural antioxidant extract of acerola on the release of sarcoplasmic calcium, myofibrillar fragmentation index (MFI) and pH of pork.

* Different uppercase letters between treatments with and without extract differ between each other and lowercase letters differ within the treatment by Tukey's test

3.3 Microstructure of meat

In samples of fresh meat, greater homogeneity was observed without the application of ultrasound (Figure 3). However, as meat exposure to ultrasound increased, the formation of cavities caused by removing fibers from small collapses generated by ultrasonic waves in the meat was noted.

3.4 Physicochemical analysis

According to the data (Figure 4), only the luminosity of the samples was not (p> 0.05) affected by the treatment applied to pork.

Capital letters represent the effect ($p < 0.05$) of Extr (Natural antioxidant extract). Lower case letters represent the effect ($p < 0.05$) of Us (Ultrasound).

On the other hand, it was possible to notice a significant effect ($P < 0.05$) of the time of application of ultrasound and extract of the interaction on the intensity of the red color (a *) of the pork loins. It was noted that the effect of the ultrasound application time varies depending on the presence or absence of the extract of the acerola residues. Figure 4, the parameter b * showed a significant effect ($p < 0.05$) for applying the extract. The samples with the application of the extract increased the index of yellow color (b*).

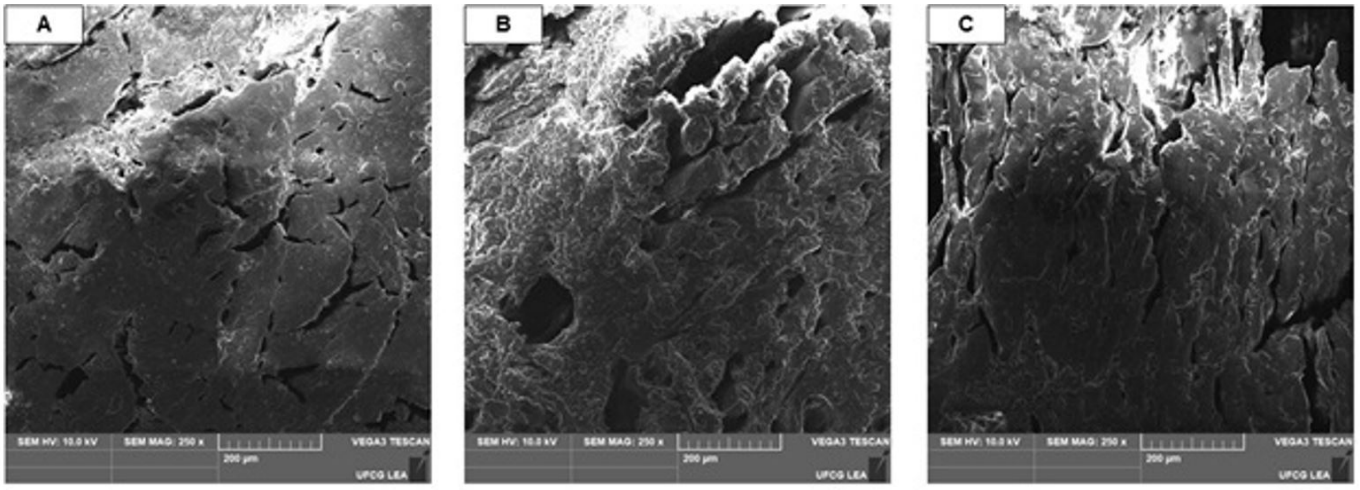

Figure 3. Scanning electron micrographs (SEM) of the different ultrasound times; (A) without ultrasound application; (B) 5 minutes of ultrasound; (C) 10 minutes of ultrasound.

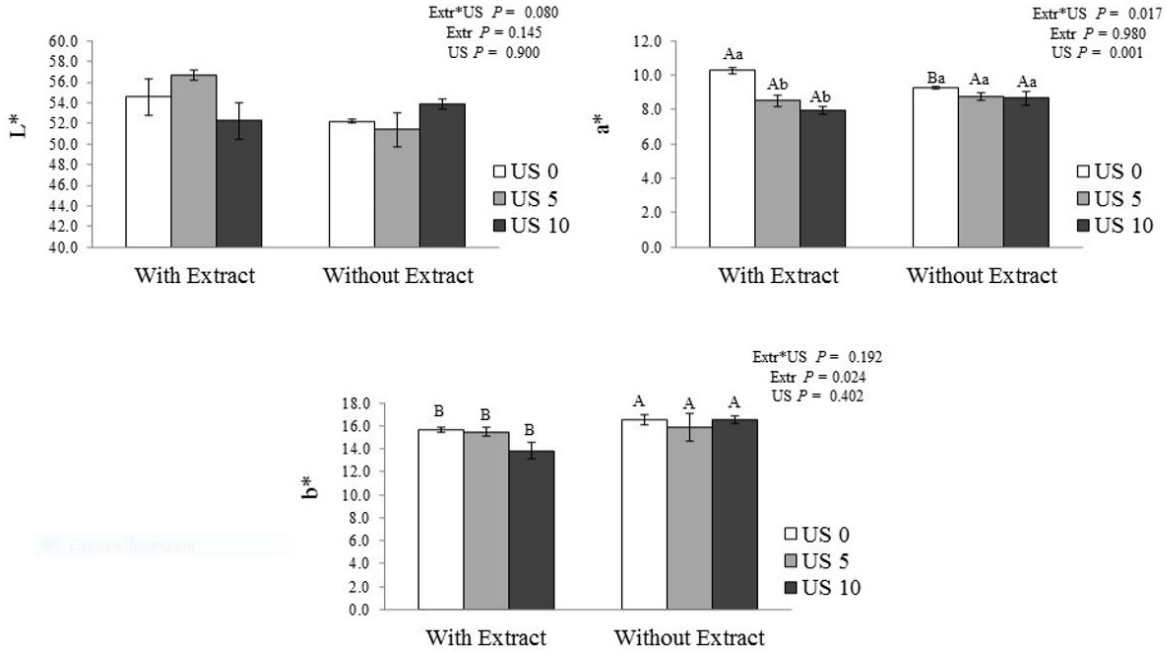

Figure 4. Effect of the application of ultrasound and natural antioxidant extract of acerola on the instrumental color of pork. * Different uppercase letters between treatments with and without extract differ between each other and lowercase letters differ within the treatment by Tukey's test

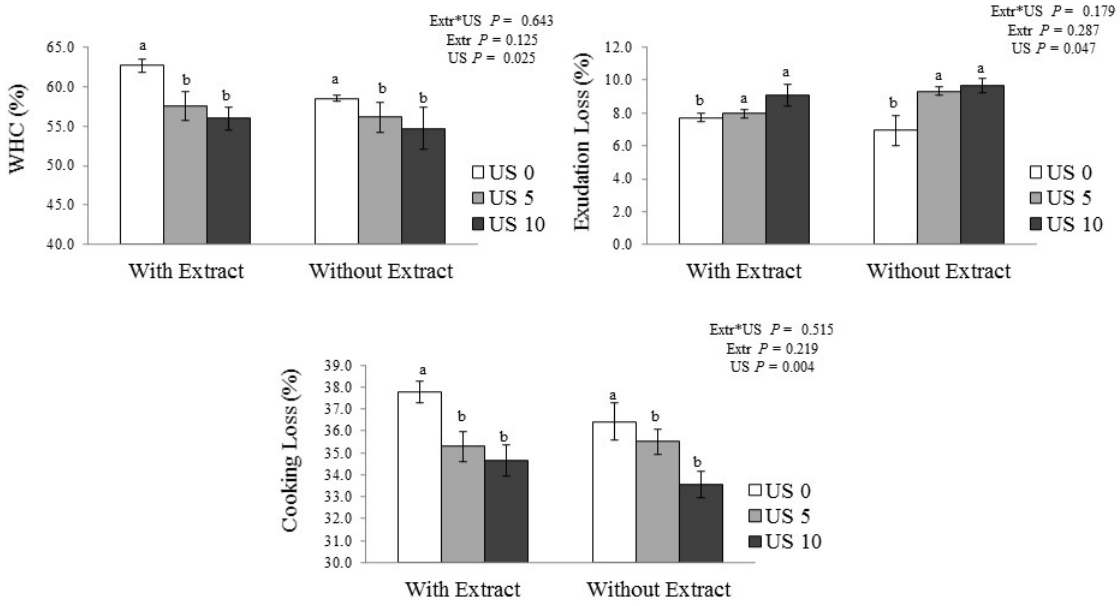

Figure 5. Effect of the application of ultrasound and natural antioxidant extract of acerola on the water retention capacity (RC%), weight loss by exudation and cooking of pork. * Different uppercase letters between treatments with and without extract differ between each other and lowercase letters differ within the treatment by Tukey's test

For the variables of water retention capacity (RC%), loss by exudation, and cooking (Figure 5), there was a significant effect ($p < 0.05$) for the application of ultrasonic waves. Regardless of applying the ultrasound (5 and 10 minutes), there was a decrease in the water retention capacity and an increase in the loss by exudation. However, for the cooking loss variable (Figure 5), there was a positive effect. After 5 minutes of ultrasound, there was a decrease in cooking loss.

Capital letters represent the effect ($p < 0.05$) of Extr (Natural antioxidant extract). Lower case letters represent the effect ($p < 0.05$) of Us (Ultrasound).

3.5 Lipid oxidation

An interaction ($p < 0.05$) of the extract and application of ultrasound is observed within 5 minutes (Figure 6). The natural antioxidant extract applied in 5 minutes of ultrasound showed better oxidative preservation concerning the other treatments.

Capital letters represent the effect ($p < 0.05$) of Extr (Natural antioxidant extract). Lower case letters represent the effect ($p < 0.05$) of Us (Ultrasound).

3.6 Multivariate analysis

The first three principal components explained 64.08% of the total variance (Table 1). The first component explained 32.25% of the variation, the second component 18.01%, and the third 13.82%. Thus, demonstrating sufficiency to explain most of the data obtained in the study.

The distribution of variables in the two-dimensional graph based on the first two components on the x-axis PC1 (32.25%) and the y-axis PC2 (18.01%). The farthest variables from the zero of the x and y axes have greater importance in the overall variation. The variables of the first two components with a load factor above 0.70 (Table 2) were the ones that most distanced themselves from the zero points (Figure 7).

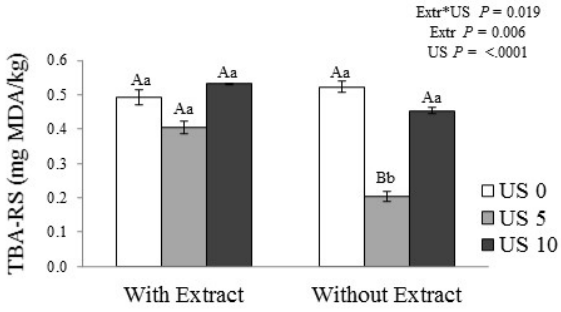

Figure 6. Effect of the application of ultrasound and natural antioxidant extract of acerola on the lipid oxidation of pork. * Different uppercase letters between treatments with and without extract differ between each other and lowercase letters differ within the treatment by Tukey's test

4 Discussion

4.1 Texture profile

The present study shows that the application of ultrasound (10 minutes) combined with the acerola residue's antioxidant extract decreased the pork meat hardness and chewiness. Some authors agree that the application of ultrasound (15 to 130 kHz) at different times changes the texture and consequently improves the meat's tenderness (Zhou et al., 2010; Stadnik & Dolatowski, 2011; Xiong et al., 2012; Pena-Gonzalez et al., 2017; Amiri et al., 2018; Xiong et al., 2020). This effect is attributed to changes in the meat's intrinsic structure, protein denaturation, myofibril degradation, and increased proteolysis (Alarcon-Rojo et al., 2019).

Table 1. Principal components (PC), eigenvalues, percentage of variance for each component and the total variance.

PC	Eigenvalues	Variance (%)	Total variance (%)
PC1	4.52	32.25	32.25
PC2	2.52	18.01	50.26
PC3	1.93	13.83	64.08

Table 2. Factor loading of the sensory attributes evaluated pig meat.

Variable	PC1	PC2	PC3
Toughness	**0.7490**	0.3990	-0.3167
Adhesiveness	0.1009	**0.8044**	-0.1691
Cohesiveness	0.3354	0.3960	**0.7407**
Elasticity	0.6601	0.1772	0.4216
Chewiness	**0.7411**	0.5196	-0.1802
Sarcoplasmic Calcium	**-0.7060**	0.1324	0.3352
Myofibrillar Fragmentation Index	**-0.7328**	0.4441	0.1421
L*	0.1743	-0.4854	0.6773
a*	**0.7801**	-0.3507	-0.0268
b*	0.2819	**-0.7310**	0.0670
Water Retention Capacity	0.2790	0.1807	0.2545
pH	-0.4587	-0.2523	-0.5382
Loss on Exudation	-0.5453	0.1795	0.2949

Note. Bold values indicates $p < 0.05$; Principal component = PC.

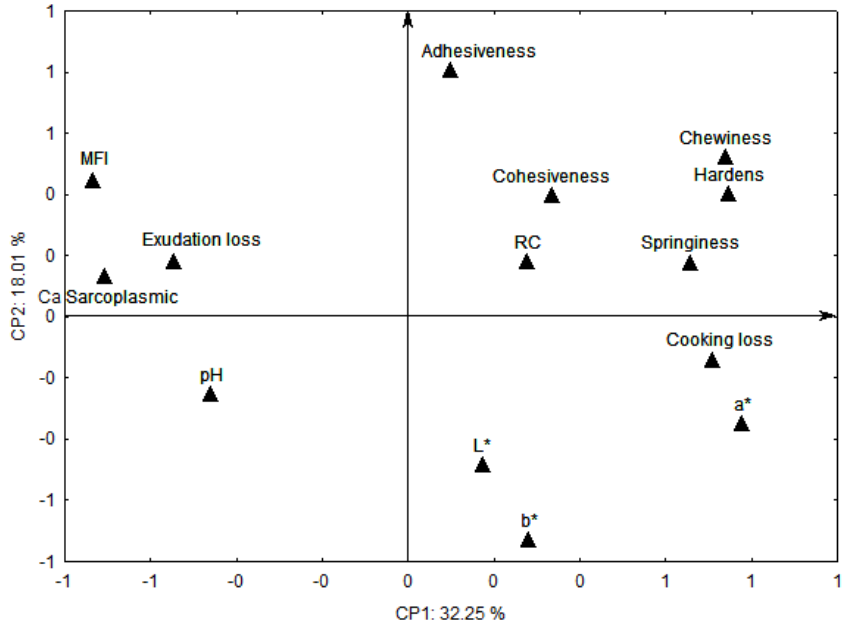

Figure 7. Two-dimensional graph for texture parameters, myofibrillar fragmentation index (MFI), sarcoplasmic calcium (Ca Sarc), color (a*, b* and L*), cooking loss, exudation loss, water retention capacity (RC) and pH. * Different uppercase letters between treatments with and without extract differ between each other and lowercase letters differ within the treatment by Tukey's test

However, there is no knowledge in the literature of the interaction of the acerola residue's antioxidant extract with the application of ultrasound. It is suggested that the extract was an adjunct as a substrate for proteolytic enzymes in improving chewiness and hardness. On the other hand, cohesiveness was modified by increasing the extract, decreasing its extension before rupture. The modification of the meat's intrinsic structure by the application of ultrasound decreased the elasticity.

4.2 Sarcoplasmic calcium, myofibrillar fragmentation index (MFI), and pH

The application of ultrasound generates vibrational energy in the water, causing the formation of cavitations and small collapses in the meat's microstructure, favoring the increase of myofibrillar fragmentation. On the other hand, cavitations damage the sarcolemma and mitochondria of muscle cells, causing an

increase in the release of sarcoplasmic calcium that activates calpains (Warner et al., 2017). Culler et al. (1978) concluded that meat samples with MFI greater than 60 are considered very tender, corroborating the present study results.

The results of sarcoplasmic calcium and MFI had a negative correlation with the hardness and chewiness of pork (Figure 2), so that the increase in MFI and the release of sarcoplasmic calcium favored the reduction of the hardness and the strength of chewiness, promoting an improvement in meat tenderness. The pH did not change due to the application of ultrasound and extract. Jayasooriya et al. (2007), in a study with beef subjected to ultrasonic waves (24 kHz, 4 minutes), observed an improvement in tenderness and no change in pH.

4.3 Microstructure of meat

The images in figure 6 can help visualize the modification caused by the application of ultrasound and its relationship with MFI and sarcoplasmic calcium (Figure 2). With the more significant breakdown of fibers and modification of the intrinsic structure of the meat. There was an increase in the MFI. On the other hand, this modification generates aggressions in cellular structures, increasing the release of sarcoplasmic calcium, consequently decreasing hardness and chewiness (Figure 1). However, this separation of the fibers increased the loss by exudation and decreased RC (Figure 5). However, the change in the meat's microstructure combined with the cooking temperature (72 °C) formed a three-dimensional network from the gelation of proteins modified by ultrasound.

Kang et al. (2017), in a study with beef cured with NaCl at different times of ultrasound, observed the formation of cavities and separation of fibers in the scanning electron micrographs, the samples submitted to ultrasonic waves had alterations in the RC and tenderness, a similar result to the present study.

4.4 Physicochemical analysis

The L* parameter was not affected by the application of ultrasound and extract. In contrast, the application of ultrasound with natural antioxidant extract preserved the red color (a *). However, the application of ultrasound (5 and 10 minutes) without the extract's presence offered a more significant loss in the red color (a *). The application of ultrasound in the meat limits the oxymyoglobin formation and delays myoglobin's formation, pigments responsible for the bright red color of the meat (Stadnik & Dolatowski, 2011).

Caraveo et al. (2015), in a study with the application of ultrasound (11 W cm −2, 40 kHz) in beef in the times (0, 60, and 90 minutes), observed a decrease in the parameter a * in the samples that had the application of ultrasonic waves in control.

The samples added with the extract of the acerola residue had a higher yellow intensity (b *). This result can be explained by the reddish-yellow color of the extract of the acerola residue. According to Munekata et al. (2015), the variation of color in meat can be related to the antioxidant extract used due to the different pigments found in extracts from fruits, seeds, and residues.

The application of ultrasound (5 and 10 minutes) caused a decrease in the trapping of water molecules, increasing the loss through exudation and decreasing the meat's capacity to retain water. The action can justify that the ultrasonic waves cause the intrinsic structure of the meat, favoring the removal of the fibers and modification of the myofibrillar proteins, contributing to reducing the trapping of water molecules in its structure (Stadnik et al., 2008).

Gómez-Salazar et al. (2018) studied rabbit meat and observed a decrease in RC and an increase in exudation weight loss after-treatment of the meat with ultrasonic waves. Studies indicate that applying ultrasound to meat without brine solution can increase loss by exudation and decrease RC (Chang et al., 2015; Li et al., 2015; Gómez-Salazar et al., 2018; Xue et al., 2018).

However, the cooking temperature used in this experiment (72 °C) combined with the application of ultrasound decreased the loss by cooking, with a change in myofibrillar structures, according to Alarcon-Rojo et al. (2015), ultrasound treatment in the cooking temperature range (50 to 70 °C) in the meat reduces water loss. Saleem & Ahmad (2016) explain that the changes caused by ultrasonic waves can alter myofibrillar proteins and consequently improve the gelation (actomyosin) trapping water molecules through a three-dimensional network. The present study suggests that this gelation had a better effect than cooking (72 °C).

4.5 Lipid oxidation

The natural antioxidant extract from the pulping of the acerola combined with 5 minutes of ultrasound showed better oxidative preservation when compared to the other treatments. It is important to note that studies with the application of ultrasound on meat observe an increase in the values of TBARS depending on the frequency, intensity, and duration of the ultrasound (Pena-Gonzalez et al., 2017; Kang et al., 2017). In the present study, it is possible to show that at 5 minutes of ultrasound, there was a better penetration of the extract and, consequently, a decrease in the TBARS values.

These oxidation processes reduce colour, texture and nutritional quality due to the degradation of fat-soluble vitamins and essential fatty acids, in addition to generating potentially harmful compounds Ribeiro et al., 2016). These reactions affect negatively the colour of both meat and meat products by favouring the formation of metmyoglobin that has a greyish brown colour (Pogorzelska et al., 2017). These alterations cause a reduction in shelf life of meat products and affect the consumer's acceptance resulting in the rejection of the product, since consumers often associate colour with freshness and meat quality (Agregán et al., 2018). Thus, animal fat replacement by other healthier sources of fat can be seen as a valuable strategy to obtain nutritionally enhanced meat products (Moghtadaei et al., 2018) but oxidative reactions can occur due to the increase of unsaturated fatty acids (Carvalho et al., 2019).

The several studies have investigated the antioxidant effect of compounds extracted from natural sources as fruit extracts, food processing residues, seeds and leaves in meat products

(Agregán et al., 2019; Carvalho et al., 2019; Fernandes et al., 2016; Munekata et al., 2016; Pateiro et al., 2018).

4.6 Multivariate analysis

The principal component technique (PC) favored identifying the variables that contributed most to the research. Among the 14 variables studied (Table 2), loss by exudation, the variable L *, pH, elasticity, and RC showed a load factor below 0.70 in the three components, evidencing its low variability in the study.

According to Cruz & Regazzi (2003), they explain that load factors above 0.70 are responsible for explaining the data's total variation. This technique reduces the study's database, preserving those with more significant variability, which better explains the research, facilitating data interpretation. Ribeiro et al. (2016) state that very close variables may remarkably correlate with each other.

The chewiness and hardness parameters positively correlate, with the decrease in hardness, consequently, a lower chewing force. It is possible to observe that MFI, sarcoplasmic calcium directly correlate with meat tenderness and increased water loss. Of the 14 variables studied, nine variables were of most significant importance in the study in the first 3 PC.

5 Conclusions

The application of ultrasound to pork (170 W, 35 kHz) in times (5 and 10 minutes) combined with marinating in the natural antioxidant extract from the acerola residue improves the quality characteristics of the meat, decreasing its hardness and chewiness, and increasing the rate of myofibrillar fragmentation and the supply of sarcoplasmic calcium to proteolytic enzymes.

The application of ultrasound (5 minutes) and natural antioxidant extract promotes the best oxidative stability. However, pork meat exposure to ultrasonic waves reduces the water holding capacity and, consequently, more significant loss through exudation. Also, the meat submitted to the ultrasound process has a lower intensity of red color, despite incorporating the acerola residue's antioxidant extract to mask this effect. The natural antioxidant extract of the acerola residue with ultrasonic waves (5 minutes) can improve the pork meat quality.

The MFI and Ca parameters showed an important increase after 10 minutes of ultrasound, consequently, these results caused a decrease in hardness and chewability. IFM and the release of sarcorplastic calcium are precursors in meat tenderization, therefore, the behavior of these variables is fully correlated with the texture profile.

The principal component analysis was efficient to discriminate and identify the characteristics with the ability to significantly reduce the number of variables evaluated experimentally.

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