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Improving the poor texture and technological properties of chicken wooden breast by enzymatic hydrolysis and low-frequency ultrasound

Juliana L. Lima[1] | **Taliana K. A. Bezerra**[1] | **Leila M. Carvalho**[2] | **Mércia S. Galvão**[2] | **Lorena Lucena**[1] | **Thayse C. Rocha**[1] | **Mario Estevez**[3] | **Marta S. Madruga**[1]

[1]Post-Graduate Program in Food Science and Technology, Department of Food Engineering, Technology Centre, Federal University of Paraiba, João Pessoa, Brazil

[2]Department of Food Engineering, Technology Centre, Federal University of Paraiba, João Pessoa, Brazil

[3]Institute of Meat and Meat Products (IPROCAR), TECAL Research Group, University of Extremadura, Cáceres, Spain

Correspondence
Mario Estévez, Institute of Meat and Meat Products (IPROCAR), University of Extremadura, Avd. Universidad, sn. 10003, Cáceres, Spain.
Email: mariovet@unex.es

Abstract: Wooden breast (WB) is a recurrent myopathy in fast-growing birds, which alters the appearance, functionality, and the texture of the breast muscle. The objectives of this study were (i) to evaluate the effect of a combined use of papain enzyme and ultrasound on the texture of WB chicken using response surface methodology and (ii) to assess the effect of marinating on the quality of WB chicken meat. Full factorial experimental design method was used to obtain the ideal conditions to soften the WB meat. The independent variables were the concentration of papain (0.1%–0.3%) and the time in ultrasonic bath (10–30 min); shear force (SF) was the dependent variable. The optimum results were obtained at a concentration of 0.2% papain and 20 min on ultrasound. Papain enzyme had a great influence on the texture of WB meat, reducing its hardness. However, the effect of the ultrasound time on the SF response was not observed. The marinated WB meat showed similar SF values and texture profile than those from normal (N) meat, with reduction in the parameters of protein and lipid oxidation. The use of papain without ultrasound bath proved to be an efficient means for improving the tenderness of WB breasts.

KEYWORDS
chicken breast, myopathy, papain, tenderization, ultrasound

Practical Application: This study shows the efficiency of the application of two technological procedures (enzymatic treatment and ultrasound) to improve the texture profile and technological properties of chicken breasts affected by the wooden breast myopathy. The economic loss caused by the world-wide occurrence of wooden breast is enormous, and the application of papain has been found to counteract the impaired properties of this abnormal chicken breasts.

Since papain is already widely used in the food industry to tenderize meat, its application in improving the quality of WB meat is straightforward.

1 | INTRODUCTION

Wooden breast (WB) is a myopathy that affects the *Pectoralis major* muscle of chicken, due to rapid growth and animal development. Hardness and the presence of hemorrhage and exudate on the surface appear as typical symptoms of WB myopathy (Petracci et al., 2019). In terms of quality, WB can affect the nutritional and technological properties of chicken breast, and its incidence leads to economic losses worldwide (Petracci et al., 2019). From a sensory point of view, one of the main problems of WB meat is the partial or integral hardness of these breasts. Therefore, there is considerable interest in developing strategies to improve the softness of WB meat.

In Brazil, the Animal Products Inspection Department (DIPOA), through Circular Letter No. 17, of December 13, 2019, classifies WB myopathy in three degrees: light, moderate accentuated, and severe (Brazil, 2019). According to the myopathy degree, chicken WB can be marketed (i) as raw fresh meat for direct consumption, (ii) used as raw meat for processed meat production, or (iii) destined for production of nonedible products (Brazil, 2019).

Marinating is a technique that involves the process of soaking or injecting meat with a solution that includes salt, phosphates, herbs, spices, tenderizers, and other additives (Istrati et al., 2012; Yusop et al., 2012). This technique results in changes in pH and leads to denaturation of connective and myofibrillar protein (Yusop et al., 2012), being frequently used for the purpose of improving the sensory and texture properties of meat (Istrati et al., 2012). Application of proteases in brine is common in different types of muscle tissues (Kang et al., 2017; Muthulakshmi et al., 2018).

The use of proteolytic enzymes, including those derived from vegetable such as papain, bromelain, and ficin, has been widely applied for meat tenderization (Gokoglu et al., 2017). Papain, a cysteine protease of vegetable origin (Bekhit et al., 2014), digests efficiently connective tissue and muscle proteins (Arshad et al., 2016). Its action has been shown to improve the toughness of spent hen breast (Muthulakshmi et al., 2018). Ultrasound of low frequency (20–100 kHz) is a noninvasive and economical technique, used mainly to improve the properties of meat without changing its quality (Pinton et al., 2019). The effects of ultrasound are due to the phenomenon of cavitation, and mechanical vibrations cause disturbances in muscle integrity and mainly modify the structure of collagen (Alarcon-Rojo et al., 2019). In addition, ultrasound can reduce the soaking time in brine, which is too long, modifying cell membranes through cavitation, improving the marinating process (Inguglia et al., 2018).

Although previous studies have provided information on changes in marinated chicken WB with phosphates and other salts (Bowker et al., 2018; Mudalal et al., 2015), the effect of marinating using proteolytic enzyme combined with the ultrasound technique in improving WB texture and quality has not yet been studied.

Therefore, the objectives of this study were (i) to apply a response surface methodology to set the optimal papain enzyme concentration and ultrasound duration to reduce hardness in chicken breasts affected by WB myopathy and (ii) to assess the impact of papain marinade at optimal conditions on the quality of WB chicken meat.

2 | MATERIALS AND METHODS

2.1 | Sample collection and breast classification

Cobb® chicken breasts, slaughter age 44 days, boneless and skinless were collected 2 h postmortem at a Brazilian slaughterhouse. The fillets were classified into two groups: wooden breast (WB) and normal breast (N). The identification of the myopathy was made by two 15 years experienced veterinary pathologists in accordance with the criteria described elsewhere (Petracci et al., 2019). The WB breasts had severe and extensive hardness throughout the pectoral muscle with the presence of hemorrhagic points on the surface, while the breast free of apparent white strips and without hardened areas or exudate on the surface was classified as normal. After collection, the breasts ($T \leq 7°C$) were packed in a polybag type zip lock bag and transported in a cool box with ice to the laboratory and stored under refrigeration ($T \leq 3°C$).

To fulfil the two objectives aforementioned, two experiments were carried out with two corresponding sample collections at the slaughterhouse. At both samplings, the entire breast fillet (*Pectoralis major muscle*) was collected from chicken carcasses.

For the first experiment, breasts were collected to carry out the full factorial experimental design aimed to

optimize the marination and ultrasound procedures to minimize the shear force (SF) values of WB chicken breasts. A total of three N and 21 WB breasts were collected. N meats were used to set the normal and desired SF reference. The WB breasts were divided into two sections. The right side of the WB breast was used for the SF analysis of the *in natura* meat (SF reference of the WB meat), while the left side was subjected to marination, following the experimental design explained in due course.

Once the first experiment was finished, a second sampling was carried out to assess the impact of the application of the optimal conditions of papain marination and ultrasound on textural properties and other quality traits of WB chicken breasts. At this second sampling, a total of three N and nine WB breasts were collected: three WB *in natura*, three WB for enzymatic marination with ultrasound, and three WB for enzymatic marination without ultrasound according to the experimental design explained in the following section. Both sides of breast meat were used for characterization, with the left side being standardized for texture and cooking loss determinations and the right side for the other analyses.

2.2 | Experimental design

For the first experiment, a 2^2 full factorial experimental design, with four factorial points (levels ± 1) and three central points (level 0), was considered to study the effect of marination process on the SF of WB meat. A total of 21 WB meats were used in this design, with three replicates for each point. The independent variables were the concentration of the papain enzyme (*E*: 0.1%, 0.2%, 0.3%; Brauzyn®100, Prozyn) and ultrasound time (U: 10, 20, 30 min). The dependent variable was SF. The enzyme concentration and ultrasonic marination time were defined based on preliminary studies conducted by our research group. In those preliminary tests, we observed a lack of effect of marinating with ultrasound (30 min) and 0% papain on SF of chicken WB, and therefore such point was not considered in our design. The response surface model was elaborated using the Equation 1:

$$\mathrm{SF} = b0 - b_1(E) - b_2U + b_3(E)(U) + 0, \qquad (1)$$

where SF is the response value predicted by the model, β_0 is the mean coefficient (or the constant) β_1, and β_2 and β_3 are the linear coefficients.

Marinating was performed by immersing the breast (≤4°C) in chilled brine (≤4°C) in a 3:1 ratio (chicken fillet:brine), according to the procedure proposed by Muthulakshmi et al. (2018). New brine was prepared for each half breast. The brine contained 5% NaCl and different percentages of the papain enzyme. The percentage of the enzyme was calculated based on the weight of the chicken breast. Each half breast was weighed, homogenized in the respective brine and vacuum packed. Then, the meat was subjected to agitation in an ultrasonic bath (modelo UltraCleaner 1400, Indaiatuba, SP, Brazil) with frequency of 40 kHz and power of 135 W RMS the temperature of 22°C, for variable times according to the experimental design conduced. After marinating, the breast fillets were dried on absorbent paper towels. N, WB, and marinated (M) WB breasts (1–7) were evaluated for SF.

After obtaining the response function (SF), analysis of variance (ANOVA) was performed and the estimated model was obtained. The treatment that presented SF values similar to those displayed by N breasts was considered the optimal (desirable shear force).

In the second experiment, the optimal marination conditions were used to assess the impact of such marination (with or without ultrasonic treatment) on the physicochemical, textural, and functional properties of chicken breasts affected by the WB myopathy. To fulfil this objective, several groups of samples were considered and all treatments were replicated three times. Normal chicken breasts (N) were compared with three groups of WB samples: those not subjected to any technological process (WB, *in natura*), WB samples treated with papain marination and ultrasound (PWB). The three replicated samples from each group were analyzed several times for each of the parameters of interest (technical replicates). The number of analyses applied to samples depended on the parameter and it is stated in due course.

2.3 | Warner–Bratzler shear force measurements

The SF was determined in the cranial region of the breasts in sextuplicate. The breasts were cut to dimensions 30 × 10 × 10 mm. Values were measured using the Texturemeter (TA.XTplus, Stable Microsystems, Godalming, Surrey, UK) with a 50 kg load cell, equipped with a Warner–Bratzler blade (HDP/WBV) and regulated with a descent and penetration speed of 100 mm/min, a penetration depth of 20 mm and a contact force of 10 g. The SF result was expressed in Newton (N).

2.4 | Physico-chemical characterization

The pH was assessed three times in each sample using a pH meter Model Q400 AS (Quimis Aparelhos Científicos

Ltda., Diadema, SP, Brazil) according to AOAC (2000). The instrumental color was measured at three points of the chicken fillet (cranial, intermediate, and caudal ends). Lightness (L^*), redness (a^*), and yellowness (b^*) values were determined on the ventral (skin-side) and dorsal (bone-side) surfaces of the cranial region of pectoralis major muscle using a digital colorimeter (Konica Minolta Chroma Meter CR-400, Osaka, Japan). The conditions were illuminant source C at a 0° standard observer. Colorimeter was previously calibrated using a white tile provided by the supplier. The moisture (No. 950.46.41), protein (No. 928.08), and collagen (No. 990.26) contents were also determined according to the methodologies described by AOAC ((2000). The assays were performed in triplicate.

2.5 | Characterization of technological properties

Cooking weight loss (CL) was determined according to Honikel (1998), with minor modifications. The samples (100 ± 2 g) were cooked in a water bath at 100°C until reaching an internal temperature of 75°C. They were then cooled and weighed again. The CL was determined by the following equation: $100 - \{[(W_i - W_f)/W_i)] \times 100\}$, where W_i and W_f are the initial and final sample weight, respectively. CL was performed in duplicate. The instrumental texture profile (TPA) was determined in the cooked samples. Before the analysis, the samples used in the determination of CL were kept under refrigeration at 4°C for 12 h. The samples were cut in 20 × 20 × 10 mm and analyzed in two texturemeter compression cycles (TA.XTplus, Stable Microsystems). The test conditions were as follows: compression of 50% of the original height, pretest and test speed of 50 mm/min, two inch diameter compression probe (P/2″), as described by Carvalho, Madruga, et al. (2020), with modifications. TPA was performed in sextuplicate.

The fractions of myofibrillary and sarcoplasmic proteins were extracted according to Zhu et al. (2011) with modifications. Solubility was determined by the formula: solubility (%) = protein concentration at 280 nm after centrifugation/protein concentration at 280 nm before centrifugation. The assays were performed in triplicate.

2.6 | Analysis of lipid and protein oxidation

Thiobarbituric acid reactive substances (TBARs) values of chicken meat were determined using the 2-thiobarbituric acid (TBA) method of Rosmini et al. (1996) and calculated from a standard curve of 1,1,3,3 tetraethoxypropane (TEP). The results were expressed as mg of MDA per kg of meat. Warmed-over flavor (WOF) was indirectly calculated from the concentration of TBARS after a cooking and storage trial as proposed by Rosmini et al. (1996) and Rocha et al. (2020) with some modifications. Briefly, the samples (30 ± 1 g) were cooked in a water bath at 100°C until reaching an internal temperature of 75°C. Subsequently, packaged samples were stored at 6°C for 48 h under fluorescent light. Then, samples were re-heated in a microwave for 4 min, and allowed to cool down at room temperature. The results were expressed as mg of MDA per kg of meat.

Protein oxidation analysis was determined using the dinitrophenylhydrazine (DNPH) method described by Ganhão et al. (2010) with some modifications. The number of carbonyls was expressed in nmoles of carbonyls per mg of protein using a hydrazone molar extinction coefficient (21.0 nM^{-1} cm^{-1}) with absorbance readings at 370 nm. Lipid and protein oxidation assays were performed in triplicate.

2.7 | Statistical analysis

In the first experiment, the effects of the concentration of papain and ultrasonic time were investigated using the response surface methodology. As aforementioned, three replicates for each point of the full-factorial experimental design were performed. The graphs of the response and desirability surface were drawn using the STATISTICA software (version 10, StatSoft Inc., Tulsa, OK, USA). In the second experiment, normal chicken breasts (N) were compared with WB *in natura* (no treatment), and WB submitted to papain marination with and without ultrasound application (MWB and PWB, respectively). Again, three true replicates of each group of samples were prepared, and each sample was analyzed repeated times (technical replicates, specified in each section) for physico-chemical parameters, texture and technological properties and oxidative stability. Data from these analyses were subjected to a Shapiro–Wilk normality test ($\alpha = 0.05$). Then, the samples were submitted to ANOVA and the means compared by the t-test ($p < 0.05$). Statistical tests, principal component analysis (PCA) of data from physico-chemical composition, technology and oxidation process of N, WB, and marinated samples were generated by the XLSTAT software (version 2014.5.03, Addinsoft, New York, USA) and graphics and graphs using the GraphPad Prism software (version 6.0 for Windows, Graphpad Software Inc., San Diego, CA, USA).

TABLE 1 Design matrix (in coded level of two variables) and response value for shear force (SF) of marinated chicken breast

Order	Marinated poultry breast	Papain (%)	Ultrasound time (min)	SF (Newton)
4	MWB-1	−1 (0.1)	−1 (10)	24.23
2	MWB-2	+1 (0.3)	−1 (10)	12.48
6	MWB-3	−1 (0.1)	+1 (30)	24.43
1	MWB-4	+1 (0.3)	+1 (30)	13.13
7	MWB-5	0 (0.2)	0 (20)	17.84
3	MWB-6	0 (0.2)	0 (20)	19.19
5	MWB-7	0 (0.2)	0 (20)	16.49
Normal breast				17.33
Wooden breast				29.52

TABLE 2 Analysis of variance (ANOVA) for full factorial experimental design

Factor	SS	Df	Mean square	*F*-value[1]	*p*
(1) Papain (%)	132.8348	1	132.8348	87.7766	0.0026
(2) Ultrasound time (min)	0.1777	1	0.1777	0.1174	0.7545
1 by 2	0.0527	1	0.0527	0.0348	0.8639
Pure error	4.5400	3	1.5133		
Total SS	137.6052	6			
R^2	0.9670				
Adjusted R^2	0.9340				

Abbreviations: Df: degrees of freedom; SS, sum of square.
[1]Test for comparing model variance with residual (error) variance.

3 | RESULTS AND DISCUSSION

3.1 | Evaluation of SF in WB samples as affected by marinating process using papain and ultrasound

The effect of marinating process on the texture of WB meat was evaluated using the complete factorial design with two factors, including the concentration of the papain enzyme (E) and the ultrasound marinating time (U). The response values, expressed as SF for each sample obtained in their respective experimental condition, are shown in Table 1. The response values (SF) were calculated from Equation 2:

$$\mathrm{SF} = 29.8199 - 59.9220\,(E) - 0.0019\,(U) + 0.1147\,(E)\,(U)\,. \quad (2)$$

The ANOVA values for the response surface are shown in Table 2. The determination coefficient (R^2 and adjusted R^2) resulting from the model was 0.9670 and 0.9340, respectively. This result shows that the model was able to provide an excellent response within the considered range. In addition, the p value observed for the effect of papain concentration ($p = 0.0026$) indicates statistical significance.

The detailed effect of the variables studied in the experiment is shown in Figure 1, in the form of a Pareto graph. This analysis revealed that the enzyme papain exhibited a very strong influence on meat tenderization, with this factor being statistically significant ($p < 0.05$). In addition, it is possible to observe that papain presented a negative effect on the response, which indicates that papain caused a dose-dependent tenderization effect on chicken meat. However, neither the ultrasound time ($p > 0.05$) nor the effect of the interaction between these two variables (papain versus ultrasound time) ($p > 0.05$) significantly influenced the softening process, within the range considered.

The response surface (Figure 2a) and the contour graph (Figure 2b) illustrate the effect of the percentage of the papain enzyme and the ultrasound marinating time on the SF of WB meat. These graphs indicate that the enzyme variable plays a very important role in the response, that is, a lower SF was achieved in the higher concentrations of papain and vice versa, while the time of marinating in the ultrasound, within the experimental conditions, had little influence on the response of the model.

Figure 2b shows that the SF in WB meats marinated with 0.10% of the papain enzyme was greater than 24 newtons, while in meats marinated with 0.30% were less than 13 newtons. The SF value obtained from meats

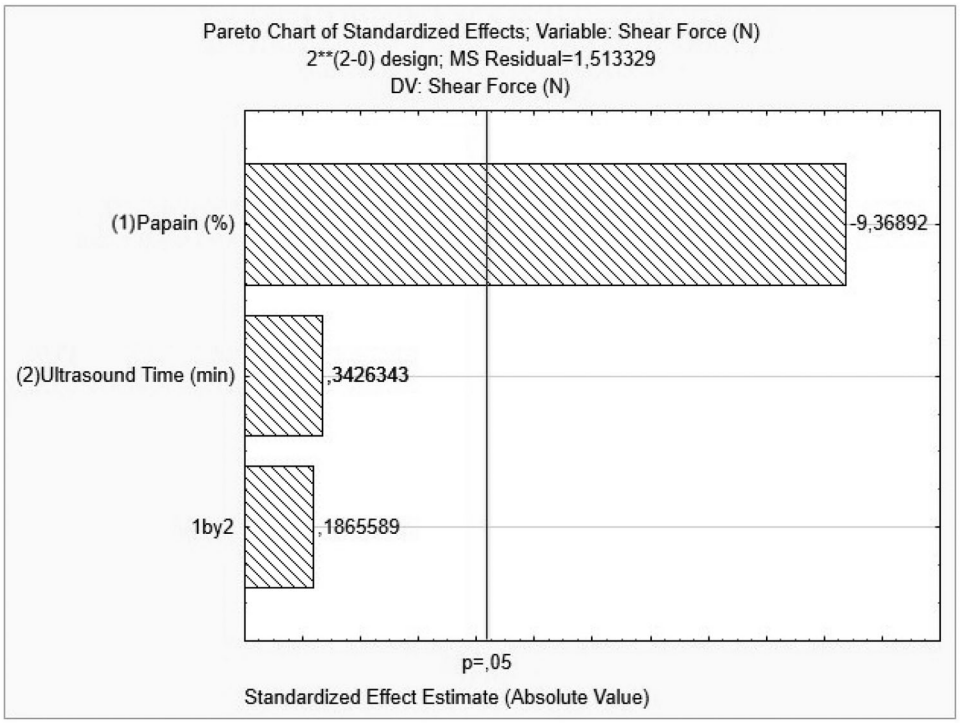

FIGURE 1 Pareto chart of standardized effects of the different variables tested in the shear force (SF) of marinated wooden breast (WB) meat

Note: the vertical line in the chart defines 95 % confidence level.

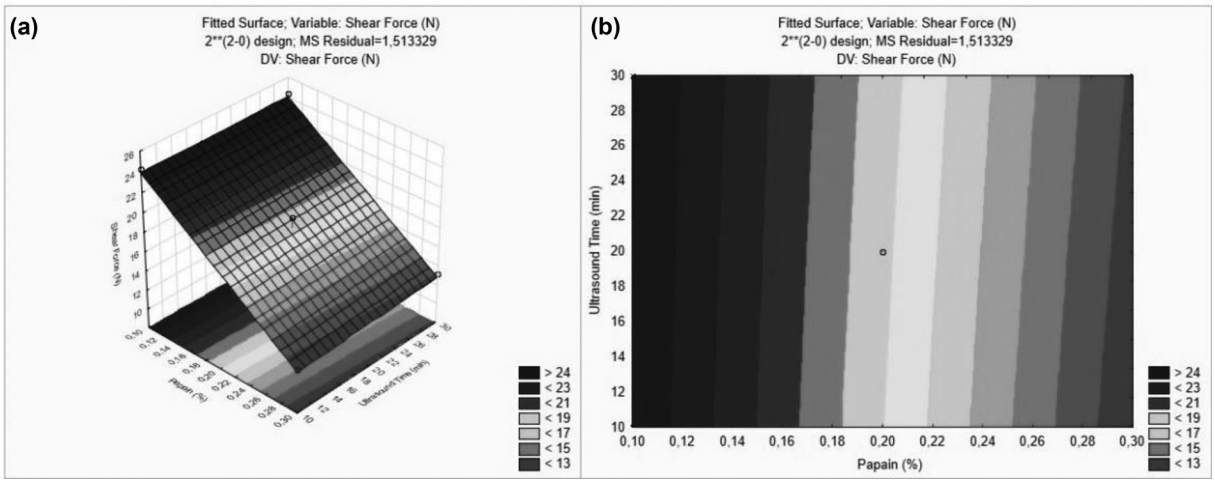

FIGURE 2 Response surface plot and contour plot showing the effects of variables on shear force

Note: (a) surface plot of shear force (SF) versus percentage of papain and ultrasound time (min); (b) contour plot of SF versus percentage of papain and ultrasound time (min).

marinated with 0.2% of the enzyme ranged from 18 to 19 newtons, regardless of the ultrasound marinating time, with these values being similar to those from N meat.

These results indicate that increasing the concentration of papain led to more intense hydrolysis in the WB meat, probably as a result of the breakdown of chemical bonds, such as the peptide bonds between the amino acids of myofibrillar proteins and collagen (Barekat & Soltanizadeh, 2019). This split resulted in a lower SF applied to the meat cut, leading to a softer meat, even with WB myopathy.

WB meat presents unwanted toughness, probably caused by chronic myodegeneration with regeneration,

accumulation of connective tissue or fibrosis (Sihvo et al., 2018). One of the characteristics of WB myopathy is the accumulation of collagen in the chicken breast, affecting partial (cranial region) or total muscle extension (Kuttappan et al., 2017; Petracci et al., 2015; Sihvo et al., 2014). However, vegetable enzymes are able to degrade connective tissue made up of 80% collagen to tenderize meat (Arshad et al., 2016). The present results corroborate this positive tenderizing effect of papain in WB meat.

Ultrasound marinating time had no effect on the SF, which is consistent with the results from Mcdonnell et al. (2014) who assessed the effect of ultrasound during the salting of pork. Ultrasound causes acoustic cavitation, a phenomenon of generation, growth and eventual collapse of the bubbles that causes thermal, mechanical wear, and chemical effects (Kang et al., 2017). Ultrasound waves are able to permeate the cell membrane and induce structural and physical-chemical changes and accelerate chemical reactions. In addition, the acoustic cavitation can induce the mechanical breakdown of myofibrillar proteins and cause protein denaturation (Carrillo-Lopez et al., 2019). These mechanical effects could have facilitated the action of papain while this action was not manifested, in the present study, in terms of tenderization (lower SF) (Marques et al., 2010).

3.2 | Physico-chemical characterization of normal (N), WB, and marinated WB samples

The results of the meat characterization are shown in Table 3. Marinated meats (MWB and PWB) have higher moisture content compared to N and WB meats. As expected, marination promoted an increase in moisture content of 2.2% and 1.9% for MWB and PWB chicken breasts, respectively, due to the uptake of brine. Regarding the moisture content of N and WB breasts no significant difference was observed, confirming the findings of other authors (Baldi et al., 2019; Carvalho, Delgado, et al., 2020). For protein content, no differences ($p > 0.05$) were observed among samples WB, MWB, and PWB, indicating that the use of papain does not cause loss of the protein fraction of WB chicken meat. However, these samples had lower protein content compared to N meat; the reduced protein content in WB meats is a result of the degenerative lesions of muscle fibers (Soglia et al., 2016).

The collagen content of marinated meat (MWB and PWB) was higher compared to N meat (Table 3). Although N meat had lower collagen content, it did not differ significantly from WB meat. Studies report higher collagen content in WB compared to N meat, as a consequence of the deposition of connective tissue in place of injured muscle fibers (fibrosis) (Soglia et al., 2016). Collagen accretion also affects the contractile properties of the muscle, decreasing the quality of the meat, by increasing toughness (Tonniges et al., 2018). WB breasts are characterized by lower protein content, and higher moisture and collagen values (Baldi et al., 2019; Carvalho, Delgado, et al., 2020; Mudalal et al., 2015). The analytical higher concentration of collagen in MWB and PWB as compared to WB samples can only be explained by the plausible effect of the marination and ultrasound application in facilitating the extractability/detection of hydroxyproline. The combination of both was particularly effective as these differences were statistically significant only between WB and PWB. Naturally, such differences, which may not correspond to an actual difference in total collagen content, were not reflected in other quality parameters, as explained in due course.

CL ranged from 17.13 to 25.89 % in N and WB breasts, respectively. Both marinated WB chicken breasts (MWB and PWB) did not differ significantly from N meat. However, WB breasts differed statistically ($p < 0.05$) from the other samples, showing higher weight loss during cooking. Xing et al. (2017) reported higher weight loss in WB meat due to the extensive loss of membrane integrity and the presence of viscous fluid on the breast surface. In contrast, the use of proteolytic enzyme papain in marinades contributes to less weight loss during cooking (Gokoglu et al., 2017). The decrease in CL usually occurs after meat improves its own water-holding capacity (Al-Hilphy et al., 2020). Latoch (2020) highlights that the marinating process, in addition to bringing benefits to the texture of the meat, makes it juicer, due to natural relationship between WHC and weight loss in meat. The greater the meat's WHC, the less weight loss during cooking (Al-Hilphy et al., 2020).

The results of pH and sarcoplasmic protein solubility did not differ ($p > 0.05$) among N, WB, and marinated (MWB and PWB) meats. However, myofibrillar protein solubility (MPs) in PWB marinated meat (84.67%) was higher compared to other samples, whereas N, WB, and MWB did not differ from each other. The greater solubility of myofibrillar proteins in sample PWB may be the result of the intense myofibrillar hydrolysis caused by the papain enzyme. According to Wouters et al. (2016), enzymatic hydrolysis considerably increases the solubility of proteins and changes their functional properties. The increase in protein solubility is related to the increase in protein–water interaction due to the increased exposure of hydrophilic groups on the surface of proteins.

TBARS of MWB raw sample were higher (0.12 mg MDA/kg) compared to the TBARS of WB raw one. No differences were observed between PWB and WB. After cooking, MWB and PWB meat exhibited lower oxidation

TABLE 3 Characterization of normal (N), wooden breast (WB), and marinated MWB and PWB chicken meat ($n = 9$, per group)

Parameter	N	WB	MWB	PWB
Moisture[1]	75.09 ± 0.31^{b}	75.64 ± 0.79^{b}	77.32 ± 0.29^{a}	77.00 ± 0.33^{a}
Protein*[1]	83.45 ± 1.50^{a}	80.26 ± 0.99^{b}	$79.05 \pm 0{,}69^{b}$	78.17 ± 1.49^{b}
Collagen*[1]	1.67 ± 0.41^{c}	$1.85 \pm 0.09^{b,c}$	$2.49 \pm 0.25^{a,b}$	2.61 ± 0.32^{a}
pH	5.98 ± 0.09^{a}	6.08 ± 0.08^{a}	6.02 ± 0.11^{a}	6.01 ± 0.04^{a}
Cooking loss[2]	17.13 ± 1.38^{b}	25.89 ± 1.87	21.01 ± 1.78^{b}	19.45 ± 2.03^{b}
Sarcoplasmic protein solubility[2]	75.90 ± 5.87^{a}	74.46 ± 0.17^{a}	77.69 ± 2.06^{a}	74.73 ± 1.63^{a}
Myofibrillar protein solubility[2]	74.77 ± 1.74^{b}	74.19 ± 3.84^{b}	76.18 ± 3.58^{b}	84.66 ± 2.77^{a}
$TBARS_{raw}$[3]	0.04 ± 0.00^{c}	0.07 ± 0.01^{b}	0.12 ± 0.01^{a}	0.08 ± 0.01^{b}
$TBARS_{cooked}$[3]	0.36 ± 0.09^{b}	1.11 ± 0.04^{a}	0.29 ± 0.03^{b}	0.34 ± 0.06^{b}
WOF[3]	1.66 ± 0.27	2.10 ± 0.18	1.64 ± 0.21	1.82 ± 0.11
Carbonyls[4]	5.98 ± 0.70^{a}	4.16 ± 0.23^{b}	$3.32 \pm 0.06^{b,c}$	2.52 ± 0.27^{c}
Instrumental color				
L^* (ventral)	56.33 ± 3.04^{b}	62.43 ± 1.75^{a}	$59.70 \pm 1.31^{a,b}$	$59.5 \pm 1.02^{a,b}$
a^*(ventral)	0.68 ± 0.57^{a}	1.65 ± 0.78^{a}	1.33 ± 0.31^{a}	2.16 ± 1.20^{a}
b^* (ventral)	7.31 ± 0.70^{a}	$5.43 \pm 1.32^{a,b}$	3.71 ± 2.08^{b}	2.95 ± 0.40^{b}
L^* (dorsal)	$55.11 \pm 0.56^{a,b}$	57.36 ± 3.45^{a}	52.48 ± 0.12^{b}	$55.89 \pm 0.50^{a,b}$
a^* (dorsal)	1.02 ± 1.42^{a}	1.02 ± 0.70^{a}	0.68 ± 0.65^{a}	0.14 ± 0.35^{a}
b^* (dorsal)	9.91 ± 0.37^{a}	9.70 ± 1.60^{a}	8.85 ± 0.85^{a}	10.09 ± 1.26^{a}

Note: MWB, marinated with 0.2% papain during 20 min in ultrasound; PWB, marinated with 0.2% papain during 20 min without ultrasound. Ventral: skin-side surface; dorsal: bone-side surface.

*Data expressed in dry base.

[1]Results expressed as g per 100 g muscle.

[2]Results expressed as percentage.

[3]Results expressed as mg MDA/kg muscle.

[4]Results expressed as nmol/mg protein.

[a,b]Mean values within the same parameter followed by different superscript letters significantly differ by the Tukey test ($p < 0.05$).

values compared to WB chicken and similar value to N meat, suggesting an improvement in the quality of chicken breasts after enzymatic treatment, irrespective of the application of the ultrasonic bath. There was no significant difference ($p > 0.05$) in WOF among the four samples. PWB had the lowest protein oxidation value, with this not being statistically different from the MWB breast. Higher total carbonyl contents were found in N chicken breast than in the manipulated WB counterparts.

Chen et al. (2020) observed a remarkable effect of cavitation on oxidative properties of meat after cooking. Natarajan and Ponnusamy (2020) reported the increase in lipid and protein oxidation in meats after the use of ultrasound. However, this behavior was not observed in our study. Oxidation occurs readily during meat processing and storage, triggering several complex chemical reactions that directly impact meat quality (Soladoye et al., 2015). WB meat has an altered chemical composition, reduced protein and higher moisture and lipid contents (Soglia et al., 2016) which make it more susceptible to oxidative processes. The values observed for the warmed-over flavor indicate that the rancid aroma would be detected in WB meat (2.10 mg MDA/kg sample), compromising the quality of the meat by reducing the shelf life, due to the formation of radicals free, hydroperoxides, malonaldehyde, and other toxic compounds. While it is not possible to ascribe the antioxidant effect of the marinade to a particular component, it is unlikely that NaCl contributed to such antioxidant effect as this salt has been found to actually, promote, both, lipid and protein oxidation (Lobo et al., 2016; Qu et al., 2020).

The instrumental color parameters presented a significant difference ($p < 0.05$) especially in the ventral region. There was no significant difference between samples for parameters a^* (ventral and dorsal regions) and b^* (dorsal region). The b^* measured in the ventral region of MWB and PWB presented lower value compared to N meat. WB breast presented higher lightness compared to N. Our findings showed no difference in L^* of chicken breast after the marinating process, indicating a similar color to N. In practice, compared to N breasts, WB breasts have higher lightness and yellowness due to the viscous liquid occurred in the ventral portion of these chicken breasts, and greater redness (a^*) due to the occurrence of hemorrhagic spots (Petracci et al., 2019).

In general, marinated meats (MWB and PWB) showed similar texture and technological properties to N meat,

in addition to a lower level of protein and lipid oxidation of cooked meat. However, as no significant differences were observed between MWB and PWB for the analyzed parameters, and because MWB uses a new technology with additional productions costs, PWB marinated meat (0.2% papain and 20 min of marination without ultrasound) seems to be the best option to improve the texture of WB meat.

3.3 | Texture properties of normal (N), WB, and marinated WB samples

The results of the SF and TPA of the chicken breasts are shown in Figure 3. The WB meat showed the highest SF differing from the N, MWB, and PWB samples. In agreement with the literature, a reduction by 40% and 48% in SF was observed in MWB and PWB, respectively, compared with WB. Dramatic reductions in SF in papain-treated meat tissues are achieved by hydrolysis of connective tissue proteins and myofibrillar proteins (Zhang et al., 2020). Muscle breakdown begins after activation of the enzyme system and includes tropamine-1, tropamine-t, desmin, vinculin, meta-vinciline, dystrophin, nebulin, and titin (Arshad et al., 2016).

In addition, SF values in marinated WB samples were statistically similar to N meat, indicating that, irrespective to the application of ultrasound, the enzymatic marinade significantly reduced the toughness of meat. Smith (2011) came to similar conclusions and highlighted that sonication alone does not alter the shear values in chicken meat.

Regarding the TPA, significant differences ($p < 0.05$) were observed among the samples evaluated, except for springiness. The cooked WB meat presented the highest hardness values among the studied treatments. However, no significant differences ($p > 0.05$) were observed among N and marinated breast (MWB and PWB).

Regarding the adhesion parameter, the marinated meats MWB and PWB had the lowest values; moreover, they differed statistically between themselves, and between N and WB meats. The lowest value observed for the PWB breast represents the greatest effort to overcome the attractive forces of this sample in contact with the probe compared to the other samples. Enzymatic hydrolysis acted mainly on the muscle surface, which led to an undesirable pasty appearance on the surface of this meat after cooking. According to Ashie et al. (2002), papain is very stable to heat and has very broad specificity, which allows them to break down muscle proteins even after cooking the meat, resulting in extreme tenderization. Similar action of papain on the surface of meat has been reported by Ionescu et al. (2008). Although, in the MWB meat, the ultrasound cavitation process seems to have allowed the action of the enzymes throughout the muscle extension and not just on the surface of the breast.

Regarding cohesiveness, N, MWB, and PWB meats did not differ statistically from each other and showed lower values when compared to those from WB meat. In addition, the marinating of the meat influenced gumminess, reducing the values observed for WB meat by 49% and 30% for MWB and PWB, respectively. WB breast showed the highest values of gumminess among treatments (47.06).

Meat toughness is highly correlated with chewability. It was observed that the immersion of WB chicken fillets in brine with papain (0.2%) reduced ($p < 0.05$) the chewability of the meat, probably due to the hydrolysis of peptide bonds provided by the action of enzymes (Arshad et al., 2016) and the greater ability to retain water in the cooking process after treatment. The lowest values of chewability were observed in the sample marinated under MWB sonication (15.63). There was no difference between N and PWB samples.

In general, the marinated samples (MWB and PWB) showed less resilience when compared to nonmarinated meat (N and WB). The samples from N and WB groups showed greater plasticity than samples from the other groups. Reduction in resilience was observed in the MWB and PWB samples, compared to untreated meats (N and WB); however, no statistical differences were found between the marinated samples ($p > 0.05$).

3.4 | Principal component analysis

PCA (Figure 4) was performed to assess the connection between all measurements of meat characterization. The first principal component (PC1) explained 56.04% of the total variation among the samples, while the second (PC2) explained 33.28% of total variability among the parameters.

Marinated meats with similar chemical parameters were positioned in nearby regions within the quadrants. PC1 separated the WB meat (in the positive side of PC1) from the N, MWB, and PWB marinated meat (in the negative side of PC1).

Texture attributes, lipid oxidation, and CL parameters were in the positive side of PC1 that were associated with the WB meat, and it was best characterized by the SF, hardness, chewiness, gumminess, cohesiveness, $TBARS_{cooked}$, and WOF. MWB and PWB meat, both submitted to the marinating process with papain, are located in the negative side of PC1, and they were associated with the chemical parameters of moisture, collagen, myofibrillar

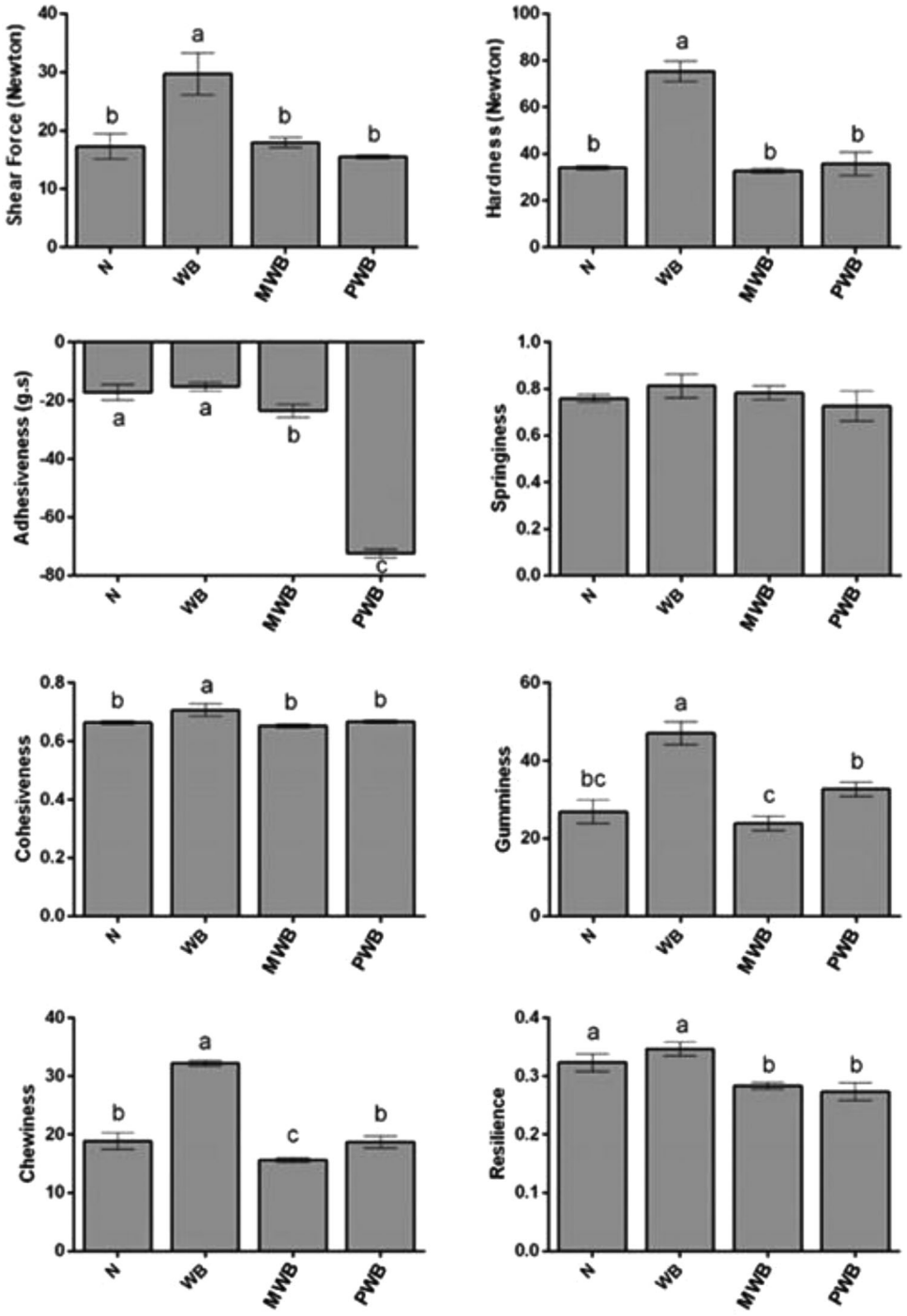

FIGURE 3 Shear force (SF) and texture profile (TPA) of wooden breast (WB), marinated WB and N chicken meat ($n = 9$, per group)
Note: [a,b]Mean values within the same parameter followed by different superscript letters significantly differ by the Tukey test ($p < 0.05$). *Note*: N, normal meat; MWB, marinated with 0.2% papain during 20 min in ultrasound; PWB, marinated with 0.2% papain during 20 min without ultrasound.

protein solubility, and $TBARS_{raw}$. N sample was positioned on the positive axis of PC2, indicating that this meat was defined by higher content of total proteins and a lower content of collagen, moisture, pH, WOF, and CL.

Thus, PCA discriminated 3 groups of chicken meats according to the quality characteristics under study. Altered texture parameters and lipid oxidation defined WB meat, while moisture and collagen characterized marinated WB meat.

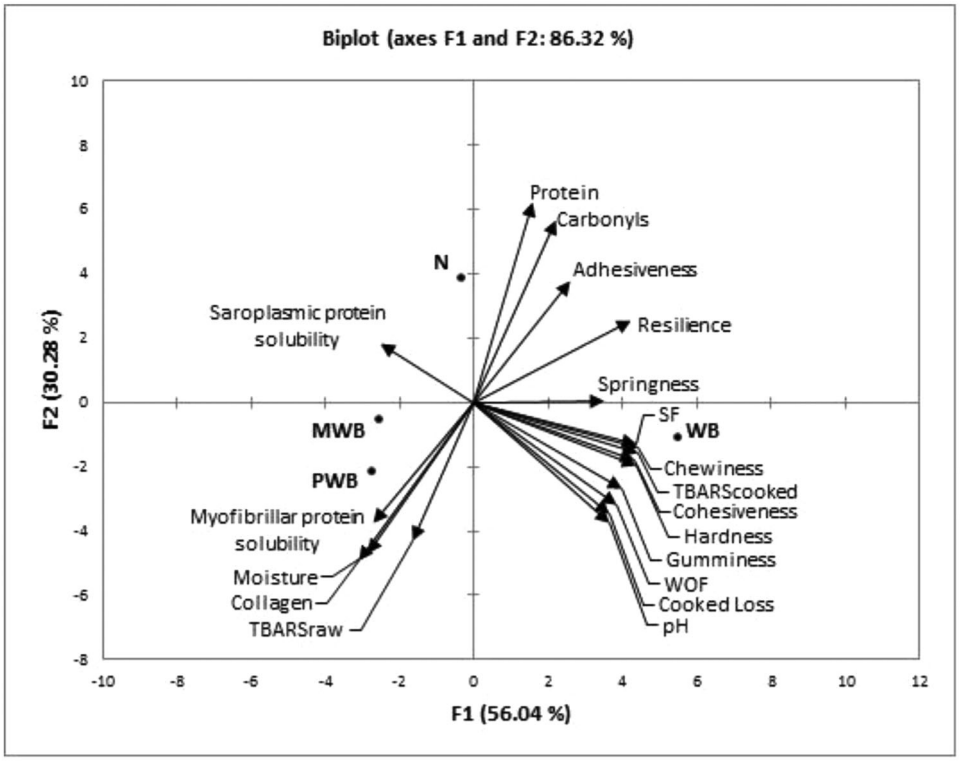

FIGURE 4 Principal component analysis (PCA) of WB, marinated WB (MWB and PWB) and N chicken meat
Note: N, normal meat; WB, wooden Breast meat; MWB, marinated with 0.2% papain during 20 min in ultrasound; PWB, marinated with 0.2% papain during 20 min without ultrasound.

4 | CONCLUSION

The use of papain enzyme in marinated WB meat had a great influence on the texture of chicken breast, reducing its hardness. Yet, ultrasonic bath time did not affect SF. The papain-marinated WB chicken meat showed similar texture and technological properties to those from N meat. Although the use of papain improved the instrumental texture indicators and the quality of WB meat, sensory tests would need to be conducted to verify if instrumental texture measures translate into an adequate sensory texture for consumers, with acceptability levels similar to N chicken breasts.

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AUTHOR CONTRIBUTIONS

Writing-original draft (Lead), formal analysis (lead), and methodology (supporting): Juliana L. Lima. *Writing review and editing (equal), methodology (supporting), conceptualization (supporting), investigation (supporting), and supervision (supporting)*: Taliana K. A. Bezerra. *Writing review and editing (equal), methodology (supporting), conceptualization (supporting), investigation (supporting), and supervision (supporting)*: Leila M. Carvalho. *Writing review and editing (equal), formal analysis (equal), and methodology (supporting)*: Mércia S. Galvão. *Writing review and editing (equal) and formal analysis (equal)*: Lorena Lucena. *Writing review and editing (equal) and formal analysis (equal)*: Thayse C. Rocha. *Writing review and editing (equal), methodology (supporting), conceptualization (supporting), investigation (supporting), and supervision (supporting)*: Mario Estevez. *Conceptualization (lead), funding acquisition (lead), investigation (lead), methodology (lead), project administration (lead), supervision (equal), validation (equal), and writing review and editing (equal)*: Marta S. Madruga.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Taliana K. A. Bezerra https://orcid.org/0000-0002-4388-4024

Mario Estevez https://orcid.org/0000-0002-8509-2789

Marta S. Madruga https://orcid.org/0000-0002-8619-3178

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