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Preparation of a cooked and salted chicken breast product using alcalase-hydrolyzed wooden breast fillets

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Abstract

Wooden breast (WB) is a myopathy characterized by functional changes and excessive hardness in chicken fillets. Alcalase, a proteinase capable of hydrolyzing meat proteins, has been shown to improve protein functionality and promote meat tenderization. This study evaluated the application of alcalase in the preparation of cooked and salted chicken breast from WB chicken fillets. WB fillets were divided into two groups: an enzyme-treated and an untreated (control) group. Enzyme-treated fillets had lower pH values, total and soluble collagen contents, collagen/protein ratio, and shear force than control. No changes in color, chemical composition, or water-holding capacity compared between groups was observed. After 2 days of storage, enzyme-treated fillets had lower lipid oxidation and total color change. Alcalase hydrolysis of WB fillets improved the tenderness and nutritional value of the cooked and salted chicken meat product. This strategy shows promise for minimizing economic losses caused by the WB myopathy.

Keywords: collagen; lipid oxidation; protein hydrolysis; texture.

Practical Application: The application of alcalase in WB fillets improved the texture of the cooked product.

1 Introduction

Wooden breast (WB), a myopathy that affects the pectoral muscle (*Pectoralis major*) of chicken, is characterized by diffuse hardened areas, whitish protuberances, vascular degeneration, mononuclear cell infiltration, interstitial inflammation, high levels of collagen, and fat fibrillation (Geronimo et al., 2022; Tasoniero et al., 2017; Sihvo et al., 2014). WB myopathy reduces water holding capacity (WHC) and alters rheological and sensory properties of chicken breast, increasing cooking loss and shear force (Xing et al., 2020; Zhang et al., 2020). Phosphate application and salt-based marinating may improve the moisture, tenderness, juiciness, flavor, and yield of poultry meat (Orel et al., 2020). WB fillets, however, have low brine absorption capacity, which, in combination with their high collagen content, particularly in the ventral portion of the *Pectoralis major*, results in low succulence (Geronimo et al., 2022; Zhuang & Bowker, 2018; Soglia et al., 2016).

Raw WB fillets have low consumer acceptance, given their pale appearance, presence of whitish protuberances, citrus-colored transudate, and hemorrhages. Thus, WB-affected muscles are generally used for the production of ground meat products and animal feed (Geronimo et al., 2022; Tasoniero et al., 2017; Kuttappan et al., 2016). The myopathy causes great economic losses in the poultry industry, estimated at $70,000 per day in Brazil in 2018 (Zanetti et al., 2018).

Proteases, such as calpains, cathepsins, alcalase, and papain, hydrolyze meat proteins, increasing meat functionality and tenderization. Treatment with proteases is a viable alternative to improve meat texture (Grau et al., 2021; Schmidt & Salas-Mellado, 2009) and still results in the generation of peptides with antioxidant properties (Xiao et al., 2022). This study aimed to treat WB-affected chicken fillets with alcalase for the preparation of a cooked and salted chicken breast product.

2 Materials and methods

2.1 Material

Seventy chicken breast (*Pectoralis major*) fillets from male and female birds (Cobb MV strain) were collected from a commercial slaughterhouse in western Paraná State, Brazil. Birds were slaughtered at 47 days of age with a mean weight of 3.141 kg. Slaughtering was performed according to conventional steps: electrical stunning, bleeding, scalding, plucking, evisceration, chilling, and mechanical separation. The project was evaluated and approved by the Ethics Committee on the Use of Animals of State University of Londrina (protocol number 10416.2018.56).

Fillets were classified as WB based on the criteria and procedures described in the literature (Tasoniero et al., 2017; Sihvo et al., 2014). Assessments involved palpation (compression test) and visual examination of the *Pectoralis major* muscle. Greater hardness, paleness, and presence of citrus-colored transudate and/or hemorrhagic lesions were considered as defining criteria.

Alcalase (Alcalase® 2.4 L FG, Novozymes; temperature range 30-65 °C, pH range 7-9) was kindly supplied by Tovani Benzaquen Ingredientes. Sodium tripolyphosphate, monosodium glutamate,

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and erythorbate were provided by Duas Rodas, and common salt (sodium chloride) was purchased from a local market.

2.2 Preparation of cooked and salted chicken breast

WB fillets were randomly divided into two groups, an enzyme-treated group and an untreated (control) group. Fillets assigned to the enzyme group were treated with brine and alcalase, whereas control fillets were treated with brine only. The brine solution consisted of 89.0% water, 2.0% sodium tripolyphosphate, 8.0% NaCl, 0.5% monosodium glutamate, and 0.5% sodium erythorbate. Brine was applied by manual injection along the dorsal axis of breast fillets without exceeding the limit of 20.0% of the meat weight, as established by Brazilian Technical Regulation (Brasil, 2003).

After brine injection, WB fillets assigned to the enzyme-treated group were subjected to alcalase hydrolysis. Each 250 g of fillet was treated with a solution containing 1.0 mL of enzyme Alcalase (enzymatic activity: 2.4 Anson Unit (AU) $gram^{-1}$ or 2.8 AU mL^{-1}) and 500 mL of water (Previous tests were performed with three enzymes, according to the literature, and the best was the Alcalase (Zago et al., 2018)). Samples were immersed in enzyme solution in sealed polyethylene bags and incubated in a water bath at 55 °C (optimal temperature for alcalase hydrolysis) for 15 min. Control fillets were immersed in 500 mL of water in sealed polyethylene bags and incubated in a water bath at 55 °C for 15 min. After this time, the water bath temperature was raised to 85 °C for at least 15 min, and all fillets were cooked until their internal temperature reached 75 °C, to ensure enzymatic inactivation and microbiological safety of the cooked product. After cooking, the water or enzyme solution was discarded, and fillets were individually sealed in new polyethylene bags. Fillets used within 24 h were kept under refrigeration (4 °C). Other fillets were stored at −18 °C until analysis.

2.3 pH measurement

pH measurements were performed, in triplicate, at 24 h after product preparation. The pH was measured in the cranial-ventral region of fillets using a potentiometer equipped with an insertion electrode (Testo 205, AG, Germany).

2.4 Color measurement

Cooked meat products (15 samples per treatment) were analyzed for color using a Minolta® CR-400 colorimeter with D65 illuminant and 10° viewing angle. Color readings were performed at three different points in the ventral region of fillets (cranial, medial, and caudal points). Results are expressed in CIELAB color parameters (L*, a*, and b*).

The total color difference (ΔE) was calculated according to the Equation 1:

$$\Delta E = [(L^*t_0 - L^*t)^2 + (a^*t_0 - a^*t)^2 + (b^*t_0 - b^*t)2]^{1/2} \quad (1)$$

where t_0 represents the fillets at cooking time zero and t represents the cooked products after 15, 30, or 45 days of storage at −18 °C.

2.5 WHC determination

The WHC of cooked meat products was determined, in triplicate, at 24 h after preparation (Troy et al., 1999). For this, 10 g of each sample was weighed, incubated in a water bath (Marconi, MA 127/BO) at 90 °C for 10 min, and cooled to room temperature. Then, samples were centrifuged (Eppendorf, 5810 R, Germany) at 8,200 × g and 4 °C for 10 min. The supernatant was discarded and the precipitate weighed. Results are expressed as percentage of water retained, calculated by the weight difference of samples before and after centrifugation, according to the Equation 2:

$$WHC = 100 - \left[\frac{(w_i - w_f)}{m}\right] \times 100 \quad (2)$$

where w_i and w_f are the initial and final weights of the sample, respectively, and m is the moisture content.

2.6 Shear force

Shear force was measured in cooked meat products using a universal texture analyzer (TA-XT_{2i}). Samples were cut against the grain into 1×1×2 cm pieces and subjected to shear testing using a Warner-Bratzler blade at a speed of 5.0 mm s^{-1}. A total of 15 samples from each treatment were evaluated, and cuts were made only in the cranial region. Results are expressed in newton (N), corresponding to the maximum force required to cut the meat transverse to the fibers.

2.7 Proximate chemical composition

Proximate composition was determined according to Association of Official Analytical Chemists (1990) methods. Moisture was determined by drying at 105 °C until constant weight and ash by incineration at 550 °C in a muffle furnace. Lipids were extracted with petroleum ether in a Soxhlet extractor after acid hydrolysis. Nitrogen content was determined by the Kjeldahl method, using a protein conversion factor of 6.25.

2.8 Total and soluble collagen contents

Total collagen content was determined as described by Woessner (1961). For this, 1.0 g of sample was added to 15.0 mL of 6.0 mol L^{-1} hydrochloric acid and hydrolyzed for 15 h at 105 °C. The hydrolyzed material was filtered and the pH adjusted to 6.0-7.0 using a 33% NaOH solution (w v^{-1}). The material was diluted to 250 mL with distilled water. Then, 2.0 mL of hydrolyzed sample and 1.0 mL of chloramine T solution were homogenized in a test tube and left for 20 min at room temperature. A 1.0 mL aliquot of perchloric acid (3.15 mol L^{-1}) was added to the solution and left for 5 min at room temperature. Subsequently, 1.0 mL of 4-dimethyl-aminobenzaldehyde was added, and the solutions were stirred and heated to 60 °C for 20 min. Samples were cooled in water at room temperature for 5 min, and the absorbance was read at 557 nm using a spectrophotometer (Libra S22, Biochrom, England). Total collagen content was determined using a standard curve of hydroxyproline solution at concentrations of 1.5 to 4.5 μg mL^{-1} and a conversion coefficient of 8.0 (Woessner, 1961).

Soluble collagen was extracted as described by Oliveira et al. (1998), with modifications. Samples (2.5 g) were homogenized for 1 min with 20 mL of deionized water and heated at 80 °C for 60 min. Then, samples were homogenized by using an Ultra Turrax equipment and centrifuged at 4,000 × g (Eppendorf, 5810 R, Germany) for 15 min. The supernatant was filtered, and 30.0 mL of 6.0 mol L^{-1} hydrochloric acid was added for hydrolysis during 15 h at 105 °C, as previously described.

2.9 Lipid oxidation analysis

Lipid oxidation was measured at 2, 15, 30, and 45 days of storage at −18 °C by the thiobarbituric acid reactive substances (TBARS) assay according to the method described by Tarladgis et al. (1960) and modified by Crackel et al. (1988). First, 10.0 g of sample was weighed and added to 15.0 mL of 7.5% trichloroacetic acid solution. The mixture was homogenized in an Ultra Turrax at 7,000 × g for 1 min, centrifuged at 6,000 × g in a refrigerated centrifuge (Eppendorf, 5810 R, Germany) at 20 °C for 10 min, and filtered through filter paper. A 5.0 mL aliquot of the supernatant was adaded with 5.0 mL of 0.02 mol L^{-1} thiobarbituric acid. The tubes were placed in a water bath at 85 °C for 35 min. Absorbance was read in a spectrophotometer (Libra S22, Biochrom, England) at 532 nm. A standard curve of 1,1,3,3-tetraethoxypropane at 0.4 to 4.40 × 10^{-9} mol L^{-1} was used. Results are expressed in mg of malonaldehyde kg^{-1} sample.

2.10 Statistical analysis

Data were analyzed using Statistica 7.0 for Windows. *Student's* t-test at the 5% significance level was applied to compare enzyme-treated and control groups. *Tukey's* test at the 5% level was used to assess differences between storage periods.

3 Results and discussion

3.1 pH, color, WHC, and shear force of cooked and salted chicken breast

The fillets used in the study had a mean weight of 364.42 ± 56.02 g, length of 187.60 ± 12.29 mm, cranial height of 37.43 ± 5.01 mm, caudal height of 27.11 ± 6.56 mm, L* value of 56.22 ± 3.25, a* value of 3.23 ± 1.55, and b* value of 6.59 ± 2.52.

It was observed that alcalase treatment acidified the chicken breast product compared with the control, resulting in lower pH ($p < 0.05$), probably due to hydrolysis and liberation of free amino acids. The difference of 0.08 in pH did not, however, influence WHC, which was similar ($p \geq 0.05$) between enzyme-treated and control groups (Table 1). It is possible that the addition of brine led to high WHC values (medium values of 95%). Raw WB fillets have a lower WHC than normal fillets, leading to water losses during processing and storage (Livingston et al., 2019; Chatterjee et al., 2016). Soglia et al. (2016) observed that marination improved the capacity of WB fillets to retain water during cooking: marinated WB fillets lost 37.8% less water during cooking than non-marinated WB fillets.

Enzyme-treated and control fillets did not differ ($p \geq 0.05$) in color parameters (L*, a*, and b*) at 24 h after treatment (Table 1). Both products were prepared from WB fillets, which are paler, redder, and yellower than normal breast fillets (Sihvo et al., 2014; Zhuang & Bowker, 2018). Cooking alters fillet color, promoting an increase in L* and b* values as compared with raw meat, masking some of the visual defects of WB fillets (Chatterjee et al., 2016). In our study, alcalase treatment did not promote changes in fillet color ($p < 0.05$) compared with the control. Furthermore, impairments in the appearance of WB meat were not mitigated by cooking, marinating, or freezing, as also reported by Zhuang & Bowker (2018) in a study comparing WB and normal fillets.

The use of alcalase was efficient to reduce the shear force of WB fillets. The maximum force required to cut meat was 17.98% lower in enzyme-treated WB fillets than in the control (Table 1). This is a relevant result, given that meat tenderness is an important quality parameter for consumer acceptance. One of the biggest problems of WB fillets is their high hardness (Mir et al., 2017). Chatterjee et al. (2016) observed that cooked WB fillets had higher shear force than normal fillets. These findings show that alcalase treatment holds promise for decreasing shear force and improving tenderness in WB meat products. The decrease in shear force is probably due to the action of alcalase on the insoluble fraction of muscle, resulting in protein and collagen solubilization (Table 2).

3.2 Proximate composition of cooked and salted chicken breast

Enzyme-treated and control WB fillets did not differ ($p \geq 0.05$) in moisture, ash, protein, or lipid content (Table 2).

Table 1. pH, color parameters (L*, a*, b*), water holding capacity (WHC), and shear force of cooked and salted chicken breasts prepared from wooden breast (WB) fillets subjected or not to enzyme treatment.

Parameter	Control WB fillets	Enzyme-treated WB fillets
pH	$6.57^{a} \pm 0.13$	$6.49^{b} \pm 0.09$
WHC (%)	$95.95^{a} \pm 0.44$	$95.99^{a} \pm 0.46$
L*	$73.16^{a} \pm 1.71$	$74.53^{a} \pm 2.43$
a*	$1.07^{a} \pm 0.60$	$1.78^{a} \pm 0.38$
b*	$13.95^{a} \pm 0.74$	$14.86^{a} \pm 1.12$
Shear Force (N)	$25.42^{a} \pm 4.92$	$20.85^{b} \pm 3.40$

Values are presented as mean ± standard deviation (n = 15). [a,b] Means within a row followed by different letters are significantly different by *Student's* t-test ($p \leq 0.05$).

Table 2. Proximate composition of cooked and salted chicken breasts prepared from wooden breast (WB) fillets subjected or not to enzyme treatment.

Parameter	Control WB fillets	Enzyme-treated WB fillets
Moisture (%)	$70.02^{a} \pm 1.65$	$71.23^{a} \pm 1.30$
Ash (%)	$1.28^{a} \pm 0.15$	$1.27^{a} \pm 0.27$
Lipid (%)	$2.91^{a} \pm 0.45$	$3.22^{a} \pm 0.58$
Protein (%)	$27.87^{a} \pm 1.04$	$28.14^{a} \pm 1.36$
Total collagen (%)	$1.31^{a} \pm 0.28$	$0.78^{b} \pm 0.07$
Soluble collagen (%)	$0.24^{a} \pm 0.05$	$0.18^{b} \pm 0.01$
Collagen/protein ratio	$4.69^{a} \pm 1.01$	$2.78^{b} \pm 0.25$

Values are presented as mean ± standard deviation (n = 15). [a,b] Means within a row followed by different letters are significantly different by *Student's* t-test ($p \leq 0.05$).

These results were expected, as alcalase is not capable of altering the macronutrient composition of fillets affected by WB; the enzyme can only modify the characteristics of proteins that are susceptible to protease activity.

Alcalase acted effectively on collagen, promoting a 1.7-fold decrease in total collagen content (Table 2). Enzyme treatment also reduced soluble collagen content, which was 0.24% in the control and 0.18% in enzyme-treated WB fillets (Table 2). Total and soluble collagen contents decreased because part of the initial collagen was lost in the exudate during cooking after being hydrolyzed and solubilized by the proteolytic activity of alcalase. The high collagen content of WB meat, compared with that of normal fillets, is responsible for the hardness of raw and cooked meat (Soglia et al., 2016; Sihvo et al., 2014). Tasoniero et al. (2019) observed a higher amount of insoluble collagen in WB meat, attributed to the high stability provided by heat-resistant intermolecular bonds. Our results showed that alcalase treatment during the preparation of cooked and salted WB fillets improves collagen solubilization.

The decrease in the shear force and collagen content of fillets suggests that alcalase acts at the molecular level in muscle fibrillar interactions, as described by Gildberg et al. (2002), who studied the proteolytic activity of alcalase on collagen. Therefore, it can be said that the decrease in total and soluble collagen levels in the presence of alcalase was due to the action of the enzyme mainly on collagen structures, improving fillet tenderness and nutritional quality. The nutritional quality of WB fillets improved because collagen has low digestibility and lacks essential amino acids (Zarkadas, 1992); alcalase activity decreased the collagen/protein ratio by 1.7-fold compared with the control (Table 2). Low nutritional quality (higher collagen/protein ratio) was also reported in fillets affected by white striping myopathy (Kato et al., 2019; Petracci et al., 2014).

3.3 Lipid oxidation and total color difference of cooked and salted chicken breast

Lipid oxidation was monitored during storage at −18 °C for 2, 15, 30, and 45 days (Table 3). After 2 days of storage, enzyme-treated WB fillets had lower lipid oxidation ($p < 0.05$) than control WB fillets. Studies have shown that some small peptides released during protein hydrolysis by alcalase have antioxidant activity (Xiao et al., 2022; Baldi et al., 2019) and these peptides might have contributed to reducing lipid oxidation in enzyme-treated fillets. There were no differences between groups at 15, 30, and 45 days of storage, showing that the protease did not contribute to the development of oxidative processes. During storage, lipid oxidation was constant in both groups, indicating that the use of erythorbate was sufficient to control oxidation.

According to Soglia et al. (2016), WB myopathy increases the amount of intramuscular fat in chicken breast fillets, replacing degenerated muscle fibers with adipose tissue via lipidosis. Such an increase, associated with the use of high temperatures during cooking, can lead to the onset of lipid oxidation, resulting in undesirable and potentially toxic compounds and alterations in the physicochemical and sensory properties of meat products (Baldi et al., 2019). Soglia et al. (2016) reported that WB fillets had higher TBARS levels than normal fillets (0.41 vs. 0.22 mg malonaldehyde kg^{-1} sample), demonstrating the importance of compounds that reduce oxidation rates, such as erythorbate.

Total color difference was measured during storage (Figure 1). Enzyme-treated WB fillets had a total color variation of 0.06 ± 0.17 after 15 days of storage, about 4 times lower than that of control WB fillets (4.21 ± 0.09). Total color difference is perceptible to the human eye at values greater than 2, depending on instrument parameters (Moarefian et al., 2013). Thus, only control products had a visually perceptible variation in color after 15 days of storage.

After 30 days of storage, enzyme-treated WB fillets had a lower ($p < 0.05$) total color difference ($\Delta E = 2.39$) than the control ($\Delta E = 4.04$), as occurred after 15 days of storage. At 45 days, however, no differences were observed between groups ($p \geq 0.05$). In general, color changes in meat and meat products are related to lipid oxidation and myoglobin processes (Zhuang & Bowker, 2018), in agreement with the lipid oxidation values presented in Table 3; enzyme-treated products showed lower oxidation after 2 days of storage and a lower change in total color.

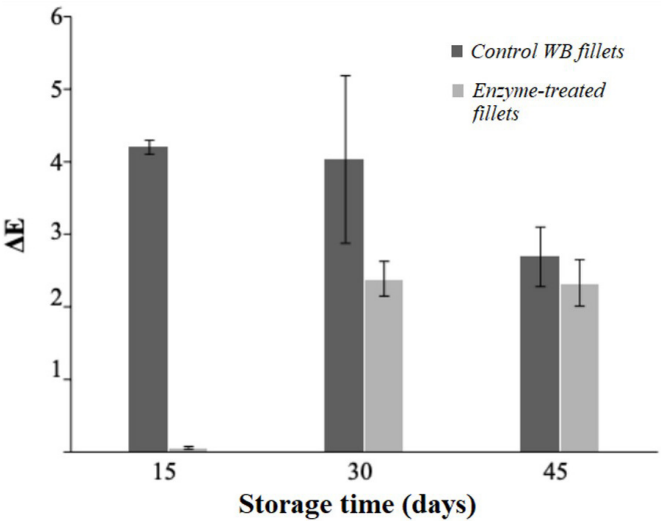

Figure 1. Total color difference (ΔE) during storage at −18 °C for 15, 30, and 45 days in cooked and salted chicken breasts prepared from wooden breast fillets subjected or not to enzyme treatment.

Table 3. Lipid oxidation during storage at −18 °C for 2, 15, 30, and 45 days in cooked and salted chicken breasts prepared from wooden breast (WB) fillets subjected or not to enzyme treatment.

Time (days)	Lipid oxidation (mg malonaldehyde kg^{-1} sample)	
	Control WB fillets	Enzyme-treated WB fillets
2	$0.33^{aA} \pm 0.00$	$0.21^{bA} \pm 0.01$
15	$0.26^{aA} \pm 0.10$	$0.30^{aA} \pm 0.02$
30	$0.27^{aA} \pm 0.06$	$0.30^{aA} \pm 0.06$
45	$0.25^{aA} \pm 0.03$	$0.29^{aA} \pm 0.03$

Values are presented as mean ± standard deviation (n = 6). [a,b] Means within rows followed by different lowercase letters are significantly different by *Student's* t-test ($p \leq 0.05$). [A,B] Means within columns followed by different uppercase letters are significantly different by *Tukey's* test ($p \leq 0.05$).

4 Conclusion

Treatment of WB fillets with alcalase improved the tenderness and nutritional value of cooked and salted WB chicken breasts. The use of enzyme-treated WB fillets for the preparation of this meat product is a viable option to reduce economic losses caused by the myopathy.

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