



Quality implications of chicken breast myopathies during refrigerated storage

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Abstract

Myopathies alter chicken breast quality through muscle structure changes such as fibrosis and lipidosis. Their presence is associated with increased pH, reduced water-holding capacity, and other impairments that may compromise shelf-life of affected fillets. This study evaluated the effects of Wooden Breast (WB) and Spaghetti Meat (SM) myopathies on chicken breast quality at four time points over refrigerated storage. Quality determinations included sensory traits (willingness to purchase, visual appearance, and odor, scored by two trained assessors), microbiological counts (total viable counts, *Brochothrix* spp., *Pseudomonas* spp., Enterobacteriaceae, *E. coli*, lactic acid bacteria), physicochemical parameters (color, pH, exudate, electric conductivity, water activity, texture), and oxidative markers (TBARS, carbonyls, relative oxygenation index). Overall, WB had more detrimental effects than SM, and both differed from normal breasts (CO). Willingness to purchase and visual appearance were lowest in WB, intermediate in SM, and highest in CO, while odor was unaffected. Microbial growth—TVC, *Brochothrix* spp., *Pseudomonas* spp., and Enterobacteriaceae—was highest in WB, intermediate in SM, and lowest in CO. Redness (a^*), pH, and exudation followed the same pattern. Texture measurements showed that WB fillets were consistently softer than SM and CO, regardless of storage time. No clear signs of oxidative deterioration were observed.

Keywords Broiler · Shelf-life · Sensory quality · Spoilage · Breast abnormality

Introduction

Myopathies in poultry, such as Wooden Breast (WB) and Spaghetti Meat (SM), are musculoskeletal diseases especially affecting the breast of commercial broilers [1]. Their incidence is substantial, but highly varies among countries, genetic strains and environmental conditions. The incidence of WB myopathy has been reported as high as 61% [2], and for SM, as high as 36% [3]. Their cause has been attributed

to secondary effects of genetic selection, which has prioritized rapid growth and, in consequence, provoked a change in the chicken's morphology and muscle characteristics [4]. Macroscopically, the WB condition is characterized by a hardened texture, while the SM myopathy involves the disaggregation of fiber bundles [1]. Even though they manifest differently, both myopathies display similar histological lesions involving inflammation, infiltration of leukocytes into the tissue, necrosis, fibrosis and lipidosis [5–7]. The implications of myopathies on meat quality have been thoroughly discussed, commonly finding increased pH, lower water holding capacity, and modified color among the most important [8–11]. Moreover, consumer acceptance and sensory attributes are significantly reduced in breasts affected by WB myopathy, with studies reporting that consumers perceive these products as unappealing and tough [12, 13]. To our knowledge, the impact of the SM condition on the purchase has not been evaluated yet.

Given this scenario, the durability of affected meat during commercial retailing conditions is a concerning issue that has not been broadly assessed. Microbiological parameters

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of breasts with WB have been studied in two instances. Gratta et al. [14] studied the microbiological shelf-life of breasts with WB stored aerobically and in refrigeration and found slower growth of bacteria (Total Viable Counts (TVC), Enterobacteriaceae, Lactic Acid Bacteria (LAB) and *Pseudomonas* spp.) on these breasts compared with normal ones over 11 days post-mortem. In contrast, Dalgaard et al. [7] used modified atmosphere packaging (MAP) of 70% N₂ and 30% CO₂ to store breasts affected with WB and found no differences between counts on either the 6th or the 8th day of storage. However, an increased diversity in microbiota, as well as promoted growth of the Enterobacteriaceae group, was observed for those breasts affected with a severe condition of WB. These contrasting results warrant further investigation. In addition, the growth of spoiler microbiota in breasts with SM myopathy was not considered in either study and has not, to our knowledge, been investigated by other authors.

Besides microbiological shelf-life, the oxidative stability of the affected meat during storage could also be a concern, since birds affected with myopathic conditions are known to suffer from muscle oxidative stress during their growth. In this line, increased markers for lipid and protein oxidation have been found in breasts with WB myopathy [6, 15, 16], which could favor further oxidative processes, shortening shelf-life.

This study aimed to assess the effects of WB and SM myopathies on the quality of chicken breasts during 14 days of refrigerated storage, through the evaluation of their sensory, microbiological, physicochemical and oxidative parameters. This study provides new comparative insights into the impact of the two most industry-relevant myopathies on chicken breast quality and shelf-life through an integrated, multi-parameter evaluation approach.

Materials and methods

Materials

Chicken breasts were obtained from whole chicken carcasses provided by an industrial slaughterhouse Barcelona region (Spain). Carcasses were transported under refrigeration prior to sample preparation and analysis. Packaging was carried out using a ULMA Smart 500 packaging system (Oñati, Spain), that sealed PP/EVOH/PE trays (Faerch A/S, Denmark) with a top film (LID830X, Cryovac Inc., Sealed Air, New Jersey, USA) under modified atmosphere conditions (30% O₂/40% CO₂/30% N₂).

For oxidative and physicochemical determinations, the following reagents and analytical chemicals were used: trichloroacetic acid (TCA), thiobarbituric acid (TBA),

2,4-dinitrophenylhydrazine (DNPH), guanidine hydrochloride, sodium phosphate salts, sodium chloride, bovine serum albumin (BSA), and associated buffer components, all of laboratory grade and purchased from Sigma-Aldrich (Merck, Madrid, Spain).

Microbiological analyses were conducted using buffered peptone water sponges (Hydrated-Sponge, 3 M, Saint Paul, USA) and culture media including Plate Count Agar (PCA, Merck), Rebecca EB (bioMérieux, Marcy-l'Étoile, France), de Man Rogosa and Sharpe agar (MRS, Merck), Streptomycin Thallous Acetate-Actidione Agar (STAA, Oxoid, Basingstoke, United Kingdom), and CHROMagar *Pseudomonas* (CHROMagar, Paris, France). Oxidase confirmation tests were performed using reagents from Scharlab S.L. (Barcelona, Spain). Peptone saline solution components were obtained from Merck (New Jersey, USA). All other analytical-grade chemicals and reagents were obtained from authorized local suppliers.

Sample evaluation, deboning and storage

A total of eighty carcasses, categorized as WB, SM and control (CO, no apparent myopathy), were selected by a trained technician on a commercial slaughterhouse and transported to IRTA (Institute of Agrifood Research and Technology) facilities under refrigeration approximately 8 h postmortem for further analysis. There, an evaluation was performed to confirm the presence of a myopathy and assess its location and severity, as follows. The presence of WB was considered moderate if hardness and pale patches with or without petechiae were observed locally on the breast, and it was considered severe if it affected at least 75% of the breast [17]. The presence of SM was considered moderate if fiber disintegration was observed locally on the breast, and mostly through palpation, and severe if the lesion was extended or if the muscle had lost its integrity, and the lesion was observable at sight [18]. In cases where breasts presented both WB and SM conditions, they were assigned with either the most severe or the most extended of the myopathies, considering, in this way, the myopathy that would alter the characteristics of both breasts in each tray the most. During myopathy assignment, carcasses were manipulated hygienically to prevent cross-contamination and three of them were discarded due to intestinal content contamination, leaving a total of 77 carcasses classified as CO ($n=23$), SM ($n=29$) and WB ($n=25$).

Carcasses were stored overnight at 1.00 ± 1.90 °C and deboned the following day (approximately 24 h postmortem). During the deboning process, gloves and surface-covering plastic used for its manipulation were discarded between samples, and cutting materials were disinfected to prevent cross-contamination. Breasts from the same animal

were packed in MAP and stored at 4.81 ± 0.75 °C in a 12-h light regime to simulate supermarket shelf conditions [19]. On days 4, 7, 11, and 14, groups of 15 breasts were retrieved and underwent further analysis. Samples on initial sampling time (considered day 0) were evaluated directly, without packaging. To assign the groups evaluated each day, the order of evaluation and deboning, as well as the severity of the myopathy, were considered to minimize their possible effects. Two extra samples (i.e. $n=17$) corresponding to the SM group were included in the sensory and physicochemical evaluations on the last day (day 14 of storage).

Sensory analysis

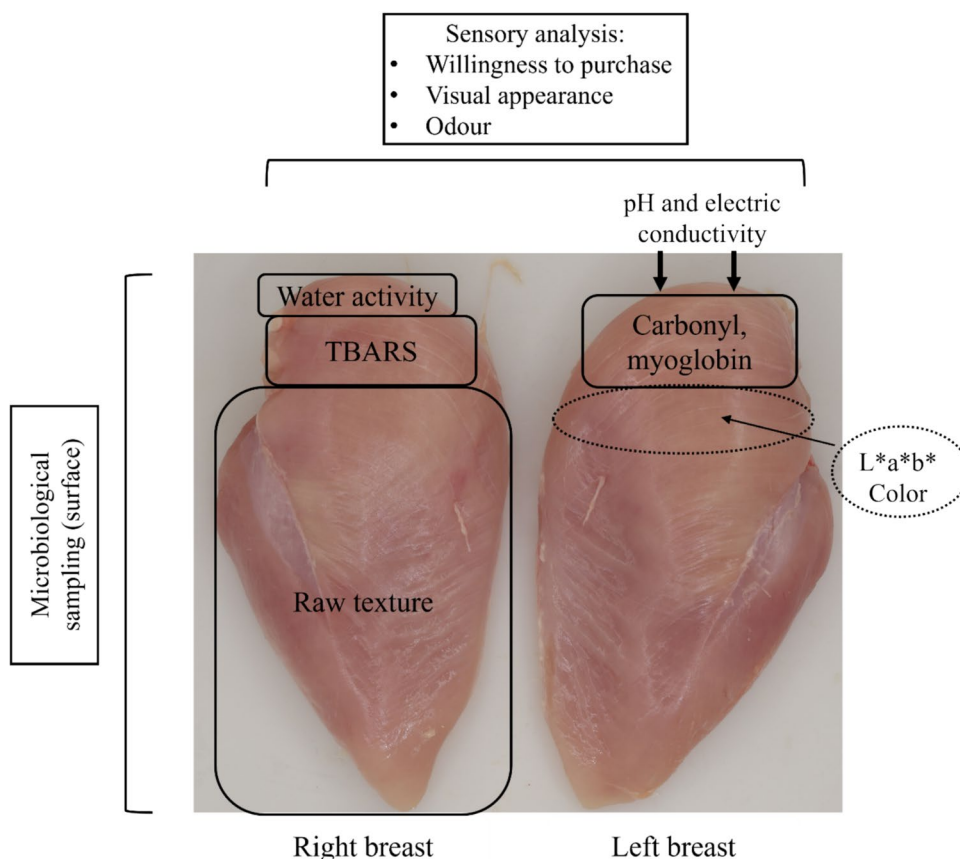
Breasts were sensory evaluated before microbiological analysis by two trained assessors. Willingness to purchase and visual appearance were assessed with the trays closed, and odor was evaluated when trays were opened for microbiological analysis. Willingness to purchase was described as the appeal of the tray to a regular consumer, considering the color of the breasts, the quantity and the color of the exudate and the presence of abnormalities such as blood points, stripes or irregular coloration. This parameter was assessed by a 5-point attitude scale (1=Definitely would not buy it; 2=Probably would not buy it; 3=Might buy it; 4=Probably

would buy it; 5=Definitely would buy it), where 3 represented the threshold, and trays with scores below that were not considered in a possible purchase. Visual appearance considered the color stability of both breasts in the tray, and paleness or irregularities negatively affected the scores. Lastly, odor scores were negatively affected by any off odors, as well as lack of the characteristic scent of poultry. Visual appearance and odor were assessed by a 5-point hedonic scale (1=Extremely disliked; 2=Moderately disliked; 3=Neither liked or disliked; 4=Moderately liked; 5=Extremely liked), where a score of 3 represented the threshold of acceptability, and breasts below that were considered not desirable.

Microbiological analysis

Approximately 10 cm² each right breast surface (Fig. 1) was sampled with a sponge pre-moistened with 10 ml of buffered peptone water. The sponge was scrubbed down 10 times on one side and scrubbed right 10 times on the other side, using moderate pressure. Then, the sponge was diluted 1/10 with peptone saline solution in a filter bag and homogenized with a Smasher™ bag blender (bioMérieux, Marcy-l'Étoile, France). Plate count enumeration considered relevant spoilage groups in poultry [19], including

Fig. 1 Scheme of breast portions used for microbiological, quality and chemical analysis. TBARS: Thiobarbituric acid-reactive substances



total viable counts (TVC, in PCA incubating 72 h at 30 °C), Enterobacteriaceae and *Escherichia coli* (in Rebecca EB incubating 24 h at 37 °C), lactic acid bacteria (LAB, in MRS incubating 72 h at 30 °C under anaerobiosis), *Brochothrix* spp. (in STAA incubating 48 h at 22 °C) and *Pseudomonas* spp. (in CHROMagar incubating 24 h at 37 °C followed by colony confirmation by oxidase test) were made. For *E. coli* and LAB, samples with counts below the plate count detection limit (<10 CFU/cm²) were assigned a value of 9 CFU/cm² for statistical analysis and graphical representation, to reflect a worst-case scenario.

Physicochemical analysis

Meat quality determinations were performed on the left breast. Figure 1 summarizes the locations and replicates performed for each analysis. The cranial region was prioritized, since this region is documented to be the most representative on myopathy occurrence [11].

Color Instrumental color was measured with MINOLTA CR-400 (illuminant D65), in three replicates on the cranial-ventral region. Measures of pH were performed in two duplicates in the cranial region using a portable pHmeter (507, CRISON, Alella, Spain) with a puncture electrode (Xerolyt type, 5232, CRISON).

Exudation To calculate the exudation percentage, breasts were weighted while deboned (day 0), and again at their respective time of storage after carefully removing excess exudate with absorbent paper. Exudation percentage was calculated as the relative difference between weights.

Electric conductivity, pH and water activity (a_w) Electric conductivity was measured using the same incisions as pH with a Pork Quality Meter (PQM-Kombi, Aichach, Germany). The replicates of each measurement were averaged. A_w was recorded using minced portions of cranial right breasts with an Aqualab 4TE (Addium Inc, Pullman, WA, USA).

Oxidative analysis Cranial portions of the breasts belonging to day 0 (CO $n=5$, SM $n=4$, WB $n=6$) and day 14 (CO $n=4$, SM $n=5$, WB $n=7$) were minced, vacuum packed in aluminum bags and stored at -20 °C until oxidative analysis (Thiobarbituric Acid-Reactive Substances (TBARS), carbonyls and relative oxygenation) was performed (Fig. 1). TBARS were calculated following Botsoglou et al. [20] with modifications. Briefly, 2–4 g of thawed minced meat were homogenized with 10 mL of distilled water in an ULTRA-TURRAX blender (30 s at 13500 rpm). 10 mL of 15% TCA were added to the mixture and incubated for

1 h at 4 °C. Following centrifugation (10000×g, 15 min at 4 °C), the supernatant was filtered and adjusted to 20 mL with 7.5% TCA. A 2.5 mL aliquot was pipetted into a screw-capped tube, adding 2.5 mL of 20 mM thiobarbituric acid solution (TBA), and the mixture was set to rest at 70 °C during 30 min in darkness. The tubes were cooled to room temperature for 30 min before absorbance at 532 nm was measured.

Carbonyl content was measured through the 2,4-dinitrophenylhydrazine (DNPH) method described in Mercier et al. [21] with adaptations. Briefly, 1 g of thawed minced meat was homogenized with 10 mL of guanidine buffer at pH 6.5 (0.19% Na₂H(PO₄)₃·7H₂O, 0.17% NaH₂(PO₄)₃·H₂O, 3.51% NaCl, 6 M guanidine HCl) with an ULTRA-TURRAX blender (30 s at 13500 rpm). After centrifugation (20000×g, 30 min at 4 °C), the supernatant was filtered. Then, two aliquots of 150 µL (one for carbonyl quantification and the second for protein quantification) were mixed with 1 mL of 10% trichloroacetic acid (TCA). After centrifugation (2200×g, 5 min at 4 °C), one pellet was treated with 1 mL of 2 M HCl and the other with an equal volume of 0.2% (w/v) DNPH and incubated in a thermostatic shaker at 25 °C for 1 h. Then, 10% TCA (w/v) was used to precipitate the samples. The supernatant was discarded after centrifugation (8900×g, 10 min at 4 °C), and the pellet was washed twice with 1:1 AcOEt:EtOH to remove traces of DNPH and residual lipids, centrifuging at 8900×g during 5 min at 4 °C, and dried using gentle N₂ steam. Absorbance at 280 nm for protein, and at 370 nm for carbonyls was measured after dissolving the pellet with guanidine buffer using an end-over agitator for 1 h, and precipitating insoluble fragments through centrifugation (360×g, 2 min at 4 °C). To determine the concentrations, a calibration line was built using BSA diluted in the guanidine buffer (0 to 1 mg BSA/mL). This method allows the measurement of carbonyl compounds by reacting them with DNPH to form stable hydrazone derivatives, which have an absorption coefficient of 21.0 mM⁻¹ cm⁻¹ at 370 nm.

Relative myoglobin oxygenation was calculated according to Richards et al. [22], after homogenizing 2–6 g of thawed minced meat in a 1:1 ratio with a buffer solution (0.892% Na₂H(PO₄)₃·7H₂O and 0.23% NaH₂(PO₄)₃·H₂O) with an ULTRA-TURRAX blender (2 min at 13500 rpm). The spectrum of the sample was recorded after centrifugation (40000×g, 30 min at 4 °C) and filtering the supernatant to remove impurities and isolate hemoglobin and myoglobin. The absorbance at the peak (575 nm) minus the absorbance at the valley (560 nm) was calculated, and the absolute value, multiplied by 100 was used for analysis. These are arbitrary units and were used to facilitate interpretation. Positive values indicated greater relative presence

of oxymyoglobin, while negative values indicated greater relative presence of deoxymyoglobin.

Texture analysis For texture analyses, portions of the right breast (Fig. 1) were vacuum packed and frozen at -20°C for 2–3 days and thawed for 24 h at room temperature. Three samples per breast condition at each time of storage were evaluated with exceptions: one sample was re-assigned from CO to SM on day 11 (the myopathy was discovered during destructive analysis, after opening the trays), and one WB sample on day 4 was discarded for texture profile analysis (TPA).

Two methodologies were applied to raw thawed meat, compression force, and texture profile analysis (TPA). To test the compression force, six parallelepipeds of 1 cm of width, 1 cm height, and 2-cm long (2–3 g) were cut and placed on a TX2 texture analyzer (Aname, Stable Micro Systems, Godalming, United Kingdom) equipped with a 30-kg load cell and a compression probe. A speed of 1 mm/s was used to record the force–deformation curve. This test analyzed the deformation of the fibers, since the parallelepipeds were placed in a support that only allowed the deformation in the fiber direction (longitudinally expansion). For TPA, six cubes of 1-cm^3 were cut in the fiber direction and compressed twice to 75% of the original height using the same texturizer and at a crosshead speed of 1 mm/s. The direction of the compression force was perpendicular to the direction of the breast fibers. Replicates that were too slimy or which curve was abnormal were discarded.

Finally, Matlab (R2023a, The MathWorks Inc. Natick, MA, USA) was used to interpret de curves and determine the maximum force, the force at 20% of the total compression, the slope (calculated as the slope of the line created between the force at 20% of the total force and the force at 80% of the total force) and the total area or total work regarding compression tests, as well as hardness, springiness, chewiness, cohesiveness and adhesiveness from the TPA test.

Statistical analysis

An ANOVA test was conducted for each variable (sensorial, microbiological or physicochemical) using breast condition (CO, WB and SM) and time of storage and their interaction as fixed effects. Severity was not used in further analysis. When the interaction was not significant, differences between time of storage and differences between breast conditions were tested separately using pairwise t-test with Holm's correction and pooled standard deviation. When interaction was present, pairwise t-tests included differences between breast condition at the same time of storage, and

differences within breast condition during subsequent times of storage. Differences between groups were considered significant when $p < 0.05$.

For parameters that included an additional procedure in the laboratory (instrumental texture and oxidative parameters), lab sampling time was included in the initial model, and means and standard deviation were corrected using least square means (LSmeans) before comparing the values.

R software was used for all the analysis (version 4.3.0, R Core Team, 2022).

Results and discussion

Sensory quality

Willingness to purchase and visual appearance were affected by breast condition and time of storage ($p < 0.01$). Supplementary Tables 1 and 2 contain their means by each category, respectively. Additionally, a tendency was observed for visual appearance to be influenced by their interaction ($p = 0.08$).

Figure 2 shows willingness to purchase (A), visual appearance (B) and odor scores (C) through storage by breast condition (CO, SM and WB). Regardless of the time of storage, the willingness to purchase scores for WB were significantly lower than those for SM, with an average difference of 0.79 points. Meanwhile, SM scores were significantly lower than CO, with an average difference of 0.94 points (Supplementary Table 1). The scores decreased over time for all groups, and on the last day they were considered unacceptable regardless of the myopathy (scores < 3). Trays with breasts affected with WB would not be considered for purchase at any time point, even on day 4 of storage, when both SM and CO scores were above the acceptability threshold. SM exceeded the threshold for a possible purchase on day 7, and meanwhile, CO breasts remained as viable options for purchase until day 11.

Visual appearance deteriorated over time with greater slope in CO and SM breasts, whereas WB scores had a more moderate slope, since their initial scores on day 4 were already lower. However, significant differences between myopathies on the same sampling day were not observed. On average, WB scores were 0.97 points below CO, whereas SM scores were not significantly different either from CO or from WB (Supplementary Table 1).

Odor was only affected by storage day ($p < 0.01$, Supplementary Table 2), and its evolution across storage time points conformed with the other evaluated sensory parameters. Specifically, day 4 had the highest scores, on day 7 or 11, punctuations dropped below the acceptability threshold (< 3), and on day 14, scores were below 2.

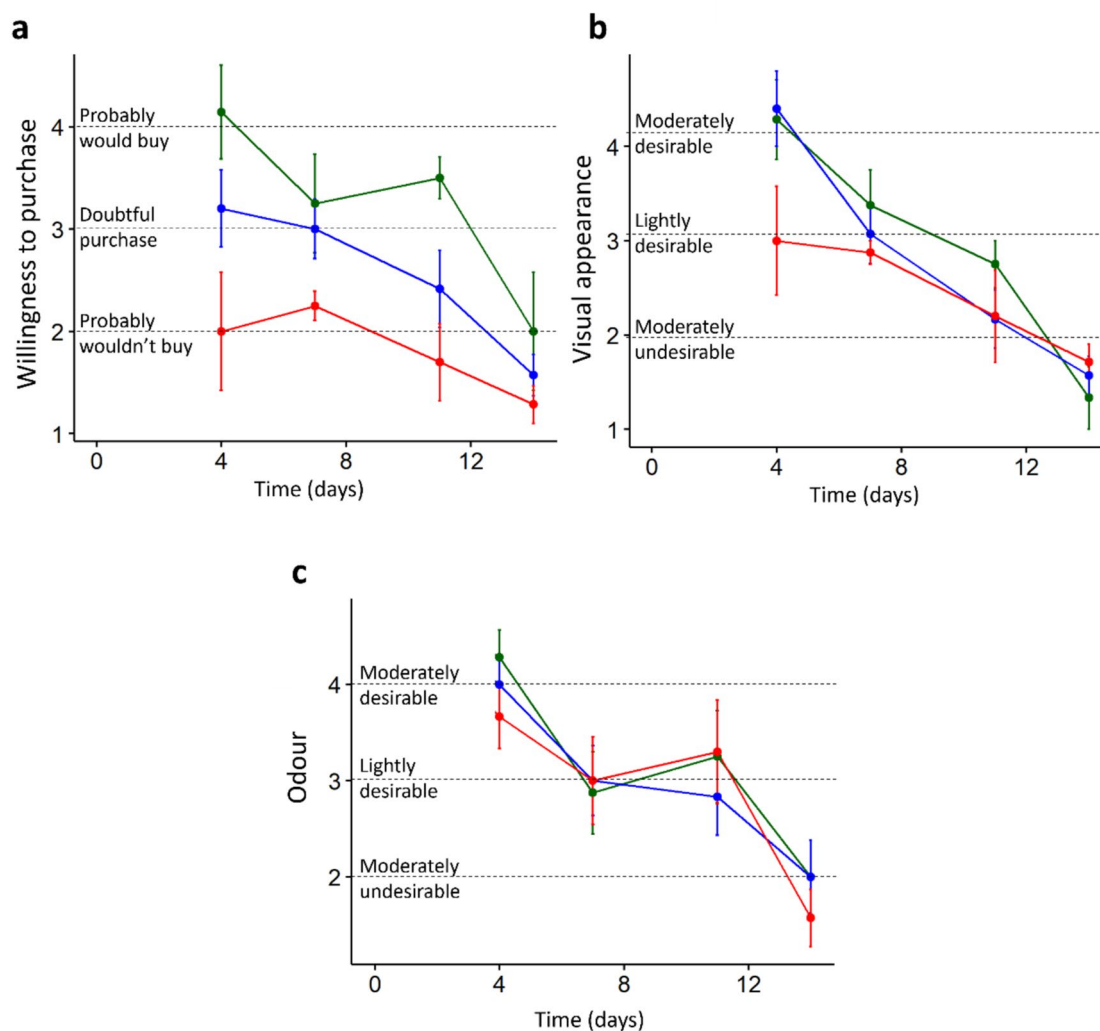


Fig. 2 Sensory attributes according to the interaction between breast condition and time of storage. Means (points) and standard deviations (bars) of (A) willingness to purchase ($p=0.44$), (B) visual appearance

($p=0.08$) and (C) odour scores ($p=0.97$). CO: control (green, $n=18$), SM: spaghetti meat (blue, $n=25$), WB: wooden breast (red, $n=19$)

Although some authors have suggested that breasts presenting myopathies would be disliked by consumers [1], few studies have thoroughly investigated the topic. Da Rocha et al. [12] investigated consumer acceptability and purchase intention of breasts affected with WB in moderate and severe degrees. Both parameters (acceptability and intention to purchase) were negatively impacted by the presence of the myopathy, with fillets scoring below the threshold of acceptability. In their case, both parameters received similar scores, in contrast with the present results, where willingness to purchase scores were lower compared to visual appearance scores within the same breast condition. A plausible explanation of this difference can be the effect of storage, since visual appearance reflects exclusively the color and the desirability of the breasts, while in willingness to purchase the whole tray was evaluated, and other factors such as exudation exerted an effect, further worsening

the punctuations. These aspects were not considered in the study of da Rocha et al. [12], as breasts were evaluated fresh and without a tray.

To our knowledge, the sensory characteristics of SM have not been investigated before. Their filamented appearance is likely responsible for decreased willingness to purchase scores, although it is only visible for severe cases. In moderate cases, SM is detected through palpation, and it is not apparent at sight. Moderate samples could be responsible for elevating the scores, ultimately placing SM breasts between CO and WB.

Microbiological quality

Figure 3 depicts the evolution of all tested microbiological groups across storage time by breast condition. Initial counts on all tested groups were comparable between breast

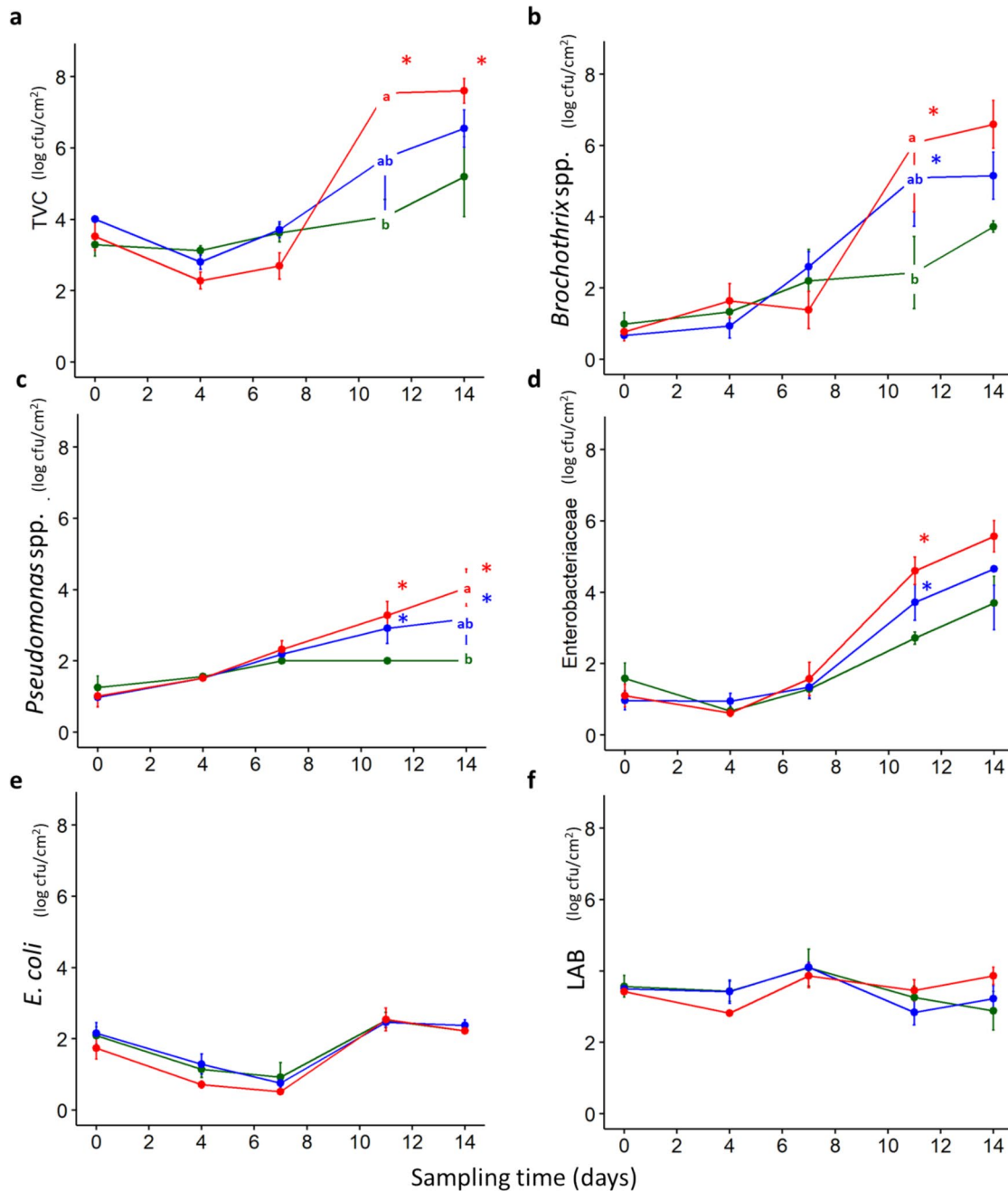


Fig. 3 Microbiological counts according to the interaction between breast condition and time of storage. Mean (points) and standard deviation (bars) of (A) Total Viable Counts (TVC) ($p=0.03$), (B) *Brochothrix* spp. ($p<0.01$), (C) *Pseudomonas* spp. ($p<0.01$), (D) Enterobacteriaceae ($p=0.04$), (E) *E. coli* ($p=0.88$) and (F) Lactic Acid Bacteria ($p=0.12$). CO: control (green, $n=23$, SM: spaghetti meat (blue,

$n=27$), WB: wooden breast (red, $n=24$). Letters represent significant differences between breast condition on the same day ($p<0.05$, pairwise t-test). Asterisks represent differences between the marked time point compared to initial sampling time (day 0) within the same breast condition ($p<0.05$, pairwise t-test)

condition. On the first days, a slight drop on microbiological counts for most groups was observed, likely as a result of a change in the microbial community composition caused by the modification of the environment (air to MAP). On day 11, breasts affected with WB myopathy reached significantly higher counts for TVC and *Brochothrix* spp. (7.53

and 6.08 log CFU/cm² on average, respectively) compared with CO counts, that remained below 5 and 4 log CFU/cm², respectively. For *Pseudomonas* spp., breasts affected with WB reached significantly higher counts on day 14 (4.04 log CFU/cm²) compared with CO breasts, that remained below plate count detection limit (<2 log CFU/cm²). Statistical

differences between breasts were not observed for Enterobacteriaceae on any storage time, although a tendency for WB counts to be higher on late storage times was observed. In addition, both WB and SM after 11 days of storage presented significantly higher counts of Enterobacteriaceae compared with the initial sampling time.

For *E. coli* and LAB, no interaction between breast condition and time of storage was found (Fig. 3), implying that their growth was not different between myopathic breasts and CO breasts. Moreover, breast condition did not affect the counts (Supplementary Table 1). However, time of storage had an impact on *E. coli* (Supplementary Table 2). Specifically, the growth of *E. coli* was inhibited during the first week (from 1.96 log CFU/cm² at day 0 to 0.74 log CFU/cm² at day 7, on average), likely by cold temperatures and MAP, but subsequently, counts increased to 2.50 log CFU/cm² at day 11 and finally reaching 2.27 log CFU/cm² at day 14, with no differences between myopathies or CO (Fig. 3). LAB counts were kept around 3.48 log CFU/cm² on average throughout storage (Supplementary Table 2).

Microbiological results reflect previous reports finding *Pseudomonas* spp., *Brochothrix* spp., Enterobacteriaceae and LAB as frequent microbiota in fresh cut meat [20, 24]. In this study, the presence of oxygen could have slowed LAB growth, according to Cha et al. [25], that found that chicken stored with 15% O₂ had lower LAB counts compared to MAP without oxygen. On the other hand, Karabagias et al. [26], that found CO₂ concentrations above 40% to significantly limit the growth of Enterobacteriaceae. In the present study, with O₂ at 30% and CO₂ at 40%, the inhibitory effect of CO₂ was observed, delaying the growth of Enterobacteriaceae and limiting LAB increase.

Another possible issue may be related to the LAB incubation temperatures, since 30 °C is known to not favor the colony formation of some psychrotrophic and cold-adapted bacteria, such as *Leuconostoc* spp., *Lactococcus* spp. or some *Lactobacillus* spp. strains, undermining LAB counts [27, 28].

In this study, myopathies were found to increase microbial growth, in contrast with previous literature [7, 14]. However, myopathies, especially WB, have been described to affect breasts increasing their pH and impairing their capacity to hold water [1, 9, 15] due to myodegeneration and replacement of muscle tissue for collagen and water [29–31]. These conditions would favor the growth of naturally present bacteria in chicken products, as observed in this study. In the study of Dalgaard et al. [7], microbiological counts in breasts with WB myopathy were investigated. In this case, breasts were stored under MAP (70% nitrogen, 30% CO₂) and at 4.0 °C. Although no differences were found with respect to unaffected breasts after 6 or 8 days of storage for either of the studied groups (TVC and

Enterobacteriaceae), they found a higher increase of Enterobacteriaceae counts between said days and a greater diversity of microbiota in WB cuts, with lower abundance of *Serratia* spp., the presence of other Enterobacteriaceae species such as *Hafnia* spp., *Aeromonas* spp. and *Shewanella* spp., and a higher proportion of non-identified species (from 0.5% in unaffected breasts to 4.5% in WB). In the present study, differences between myopathies were found after a longer storage time. In another study, Gratta et al. [14], found breasts affected with WB to have lower counts than unaffected breasts for most studied groups (TVC, *Pseudomonas* spp., Enterobacteriaceae and LAB), reaching 7 log CFU/g at a posterior time. In this case, breasts were stored in air, which might be the cause of the contradictory results. Another factor that might impact the results is the sampling methodology. Increased exudation in myopathic breasts might carry microorganisms from the surface of the meat to the exudate, underestimating counts when sampling the meat, but not when the exudate is analyzed. In this study, in contrast to Gratta et al. [14] and Dalgaard et al. [7], a sampling of the surface with a sponge was employed, which might have included more representation of exudate.

The differences between breast conditions found in TVC, *Brochothrix* spp., *Pseudomonas* spp. and Enterobacteriaceae counts were observed on days that typically surpass shelf-life of poultry products [32]. However, the higher counts observed in myopathic breasts, especially WB, are relevant since they indicate a potential reduction of the shelf-life of breasts with this myopathy, especially under storage at abusive temperature or higher initial load. This could implicate an increase in food waste and an additional cost for the food industry, and is especially meaningful under climate change conditions, where the cold chain is increasingly at risk [33].

Physicochemical quality

Meat quality parameters were not affected by the interaction between breast condition and time of storage (Table 1). However, the redness index (a^*), pH and exudation were influenced by the presence of myopathies and storage days separately (Supplementary Tables 1 and 2, respectively). Moreover, electric conductivity and yellowness index (b^*) were affected by storage days but not by myopathy (Supplementary Table 2), and water activity and lightness (L^*) were not impacted by either effect.

Focusing on instrumental color variables, regardless of myopathy, breasts became less yellow over time. When all breast types were pooled, initial b^* was significantly higher than the rest of the values (11.36 in day 0 vs. 9.60 on average from 4 to 14, Supplementary Table 2), which correlates well with the sensory scores of visual appearance. Red values (a^*) increased slightly on days 4 and 7 to decrease again on

Table 1 Least square means of meat quality parameters according to the interaction between breast condition (B) and time of storage (T). CO: control ($n=23$); WB: wooden breast ($n=25$); SM: spaghetti meat ($n=29$)

	Breast condition	Time of storage (day)					<i>p</i> -value			RMSE
		0	4	7	11	14	B*T	B	T	
<i>L</i> *							0.19	0.11	0.65	2.145
	CO	57.49	57.49	55.26	57.32	57.80				
	WB	57.69	59.85	58.53	57.74	58.01				
	SM	58.61	58.31	56.50	57.08	57.17				
<i>a</i> *							0.81	0.02	0.02	0.760
	CO	-0.60	-0.35	0.39	-0.07	-0.39				
	WB	-0.38	0.72	1.00	0.70	0.42				
	SM	-0.53	-0.39	0.27	0.02	0.22				
<i>b</i> *							0.28	0.20	0.01	1.297
	CO	11.20	9.61	8.96	9.42	8.51				
	WB	11.20	9.70	9.48	10.20	10.30				
	SM	11.80	9.11	9.22	9.90	10.10				
pH							0.20	<0.01	<0.01	0.111
	CO	5.80	5.71	5.66	5.60	5.68				
	WB	5.97	5.88	5.91	5.85	5.90				
	SM	5.95	5.78	5.75	5.69	5.69				
Exudate ^a (%)							0.17	<0.01	<0.01	3.797
	CO ($n=17$)	-	4.47	6.30	7.67	6.43				
	WB ($n=19$)	-	9.23	9.12	15.70	17.00				
	SM ($n=25$)	-	7.13	8.20	8.95	12.40				
Electric conductivity							0.43	0.69	<0.01	2.086
	CO	9.66	15.20	16.20	16.40	17.00				
	WB	8.49	12.70	15.70	16.70	17.50				
	SM	7.75	13.70	16.70	16.00	16.80				
Water activity							0.82	0.14	0.07	0.0024
	CO	0.987	0.986	0.988	0.988	0.988				
	WB	0.989	0.987	0.988	0.988	0.990				
	SM	0.988	0.986	0.989	0.986	0.990				

RMSE root mean square error; ^aThe exudation percentage was calculated by weighing deboned chicken breasts before and after storage, with the difference in weight indicating the exudate loss

the next days (Supplementary Table 2), with values on day 0 (average of -0.49) being significantly different from values on day 7 (0.50, on average). Moreover, redness index had greater overall values for WB compared to CO (0.41 and -0.24, on average, Supplementary Table 1). These results match previously reported data, that finds increased redness in breasts with WB, due to their petechia and inflammation [1, 11, 34, 35].

The values of pH of WB fillets were higher, on average 0.20 points, than CO (Supplementary Table 1), which is a common pattern that has been observed in previous studies [1, 7, 11, 36]. For SM, although values were slightly higher on the initial day (not significant, $p>0.05$), they dropped with time and were comparable to CO samples (Table 1). When pooling all breast types, a pH drop was observed with time, with values on day 0 being significantly higher than values on day 11 (5.91 and 5.72, respectively, Supplementary Table 2). This phenomenon could be associated with the dissolution of CO₂ from the MAP in the meat [37].

Exudation of CO breasts was on average of 7.96% lower than those with WB and 3.54% lower than those with SM, with significant differences between breast myopathies and CO ($p<0.01$, Supplementary Table 1). In all cases, exudation increased with time (Supplementary Table 2). By day 11, the exudate in WB trays reached values exceeding 15% of the fillet's initial weight (Table 1). These findings are in accordance with studies showing greater free water and extra-myofibrillar water in WB tissue [29, 38] and SM tissue [39]. Wang et al. [8] also found greater drip loss in WB fillets, that further increased when the condition was severe or in combination with other myopathies. As suggested in Section 3.1, this can explain differences between willingness to purchase and visual appearance scores. Other measured parameters such as water activity or electric conductivity were not able to detect these differences in water holding capacities. In this case, electric conductivity values were seen to change by storage days, and water activity only showed a tendency to increase by time of storage

(Supplementary Table 2). As proposed in Muñoz-Lapeira et al. [11], a large sample (such as the whole breast) might be necessary to gather sufficient representation when dealing with water holding evaluation in poultry, since these products do not usually present high exudation, and conventional parameters result in small values in this matrix.

Table 2 presents the means of texture parameters by breast condition and time of storage. Two texture tests were conducted: compression test and texture profile analysis (TPA). The compression test measured the structural integrity or rigidity of the muscle fiber alignment, since in this test the sample was loaded into a cell that allowed for its

longitudinal movement along the fiber axis [40]. On the other hand, TPA measured multiple texture attributes by simulating the action of chewing. Neither of the evaluated parameters were affected by the interaction between these factors; however, they showed differences between breast conditions (Supplementary Table 1), and between storage days (Supplementary Table 2).

Regarding compression tests, maximum force showed a tendency to be higher in CO breasts than in WB breasts, with SM fillets showing intermediate values ($p=0.10$). The work required to compress the tissue (area under the curve) was also significantly lower in WB compared to CO or

Table 2 Least square means of texture parameters according to breast condition (B) and time of storage (T) corrected by the day when the measurement was taken. CO: control ($n=14$); WB: wooden breast ($n=15$ for compression test and $n=14$ for TPA); SM: spaghetti meat ($n=15$)

Breast condition	Time of storage (day)					<i>p</i> -value			RMSE
	0	4	7	11	14	B*T	B	T	
Compression test									
Maximum force (kg)						0.48	0.10	0.05	0.679
CO	9.08	4.74	4.99	6.56	4.73				
WB	6.26	3.88	3.77	5.60	4.86				
SM	8.13	4.72	4.28	5.20	4.94				
Force at 20% ^a (kg)						0.23	0.86	<0.01	0.094
CO	2.03	1.75	1.79	1.63	1.67				
WB	1.89	1.63	1.8	1.53	1.85				
SM	1.97	1.53	1.84	1.66	1.80				
Slope (kg/s)						0.28	0.01	<0.01	0.221
CO	2.12	1.58	1.61	2.33	1.64				
WB	1.99	1.85	1.64	2.66	1.88				
SM	1.93	1.81	1.30	1.82	1.73				
Total area (kg*s)						0.25	0.02	0.05	2.990
CO	38.8	23.2	25.9	29.5	20.8				
WB	29.6	16.6	17.5	21.0	24.2				
SM	35.1	20.7	23.8	24.9	26.8				
TPA test									
Hardness						0.28	0.30	0.36	0.731
CO	6.67	6.37	6.38	7.93	5.41				
WB	5.55	5.02	5.82	6.11	6.94				
SM	5.74	5.43	6.44	6.30	5.75				
Springiness						0.11	0.06	<0.01	0.013
CO	0.251	0.237	0.261	0.302	0.253				
WB	0.228	0.250	0.237	0.247	0.259				
SM	0.226	0.238	0.26	0.278	0.255				
Chewiness						0.25	0.07	0.03	0.092
CO	0.485	0.284	0.380	0.666	0.290				
WB	0.330	0.188	0.246	0.285	0.378				
SM	0.338	0.235	0.407	0.447	0.298				
Cohesiveness						0.33	0.06	0.06	0.024
CO	0.295	0.182	0.214	0.264	0.194				
WB	0.252	0.152	0.176	0.174	0.204				
SM	0.254	0.178	0.231	0.239	0.192				
Adhesiveness						0.11	0.30	0.02	0.015
CO	0.032	0.054	0.042	0.038	0.079				
WB	0.031	0.046	0.037	0.033	0.054				
SM	0.032	0.049	0.090	0.025	0.050				

RMSE root mean square error; ^a Force at 20% was calculated as the force employed at the 20% of the compression test

SM ($p < 0.05$). However, the force at 20% of compression – which reflects the resistance of the muscle fibers independent of other structural elements – and the slope of the curve (an indicator of the elasticity), was not affected by the presence of myopathies.

These findings contrast with previous studies that have consistently reported increased maximum compression force in WB samples using raw breast fillets [8, 40–42]. Moreover, several authors have found that compression force increases with severity of WB [2, 43]. In the present study, the opposite was observed, likely due to the freezing and thawing of samples prior to analysis. Support for this hypothesis comes from Muñoz-Lapeira et al. [11], who reported reduced Warner–Bratzler shear force in WB samples compared with unaffected fillets after thawing and cooking, indicating that frozen storage could have a possible effect on texture. This textural softening may be linked to the altered water-holding capacity typical of WB tissue [15, 44]. The migration of intracellular water during freezing and further drip loss during thawing could reduce tissue resistance by depleting water and thus reducing juiciness. Additionally, the formation of ice crystals during freezing is known to disrupt muscle fibers – already structurally compromised in myopathies [5, 15] – further weakening the tissue. This mechanism could explain the decreased resistance in WB. However, if fiber rupture were the primary factor, we would also expect changes in the force at 20% compression, which was not observed. This metric isolates fiber contribution, excluding structural elements such as collagen [40].

Pascual Guzmán et al. [45] compared CO, SM and WB fillets and found no significant differences between SM and CO, which aligns with the current results. Additionally, the effect of storage time was also observed in the present study: maximum force, force at 20% compression and work (area under the curve), all decreased with time, consistent with proteolysis during meat aging [46].

Regarding TPA, chewiness and cohesiveness values were lower when breasts were affected with WB ($p < 0.1$,

Supplementary Table 1), while hardness, springiness and adhesiveness did not differ significantly among WB, SM and CO. The reduction in chewiness and cohesiveness suggests that WB fillets were softer, which is consistent with the compression test results. However, the absence of differences in hardness contrasts with findings from both the compression test and previous studies [40, 43].

These discrepancies can be attributed to differences in the experimental setups of both tests. In TPA, the deformation occurs without lateral restriction, allowing the muscle fibers to expand both transversally and longitudinally, unlike the compression tests, where deformation is longitudinal. As a result, the two methods capture different mechanical responses of the muscle tissue.

Since TPA is typically conducted on cooked samples, there are limited studies applying it to raw meat. Among those, Zhang et al. [43] reported increased chewiness in WB samples, which contradicts the present findings. In contrast, Campo et al. [40] observed decreased cohesiveness in WB compared to normal, aligning with the present results. However, both studies reported increased hardness in WB fillets, which was not observed in the present results.

Table 3 presents the means and standard deviations of oxidative parameters according to breast condition and time of storage. No significant interaction between these two factors was observed. When averaged across storage days, TBARS values were affected by breast condition ($p < 0.05$), though no significant pairwise differences emerged between myopathies or CO. Protein carbonyls were unaffected by breast condition, while a tendency for increased relative oxygenation in SM samples was detected ($p < 0.05$; Supplementary Table 1).

When averaged across breast conditions, storage time had a significant effect on lipid oxidation: TBARS increased by a mean of 31.5 μg MDA/kg from day 0 to day 14, and relative oxygenation values decreased over time. In contrast, carbonyl levels remained stable (Supplementary Table 2). TBARS values below 0.1 mg MDA/kg of meat are generally considered

Table 3 Least square means and standard deviation of oxidative parameters according to breast condition and time of storage corrected by the day when the measurement was taken. CO: control ($n=9$); WB: wooden breast ($n=13$); SM: spaghetti meat ($n=9$). TBARS: Thiobarbituric acid-reactive substances

Time of storage	Breast condition			<i>p</i> -value		RMSE	
	CO	WB	SM	B*T	B	T	
TBARS (μg MDA/kg)				0.59	0.01	<0.01	20.344
Day 0	3.83	20.28	27.33				
Day 14	42.61	39.81	63.23				
Carbonyls (nmols/mg of protein)				0.32	0.64	0.80	0.997
Day 0	4.17	4.61	4.07				
Day 14	5.02	3.70	4.21				
Relative oxygenation ^a				0.27	0.08	0.20	0.861
Day 0	-0.32	0.25	1.68				
Day 14	-0.47	-0.48	-0.01				

^a Relative oxygenation was calculated subtracting the absorbance at 560 nm from the absorbance at 575 nm and multiplying by 100

low in poultry, indicating minimal lipid oxidation [47]. However, the decline in relative oxygenation suggests progressive oxidation of myoglobin during storage, with more oxidized forms present at day 14 – negative values imply greater deoxymyoglobin relative to oxymyoglobin.

The elevated relative oxygenation observed in SM samples compared to CO and WB may be related to structural changes associated with the myopathy. Muñoz-Lapeira et al. [48] reported greater levels of oxygenated myoglobin in SM meat, attributed to disorganized muscle fibers that increase exposure to oxygen due to their disrupted structure.

These findings contrast with other studies that report increased oxidative markers in myopathies. For example, Rocha et al. [16] observed increased TBARS and carbonyls in WB over 90 days of frozen storage. Similarly, Soglia et al. [6] found higher TBARS and carbonyl levels in WB compared to unaffected breasts. Moreover, fillets affected by WS alone, and in combination with WB, also presented increased carbonyl content. Conversely, Tasoniero et al. [39] reported similar progression of muscle degradation in SM and unaffected samples over seven days of storage, supporting the idea that SM may not induce oxidative changes to the same extent as WB or WS.

Conclusion

The presence of myopathies in chicken breasts – particularly WB and, to a lesser extent SM – substantially affected the tested sensory, physicochemical, and microbiological parameters during 14 days of refrigerated storage. This must be considered in retail selling, and processed meat applications, since myopathies might alter the products, potentially implying impaired durability, higher pH, exudation, altered colour, and microbial growth, compared with unaffected meat. Commercialized products including myopathies should be tested, and their expiration date adjusted if necessary. Otherwise, these alterations can entail economic losses for producers and contribute to food waste, especially under climate change conditions. Conversely, markers of oxidative degradation were not obviously influenced by myopathy's presence, and the texture of raw meat was not affected in an obvious and deleterious way.

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Declarations

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