Effect of freezing on the quality of breast meat from broilers affected by White Striping myopathy

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ABSTRACT The aim of this study was to evaluate possible changes in the quality of chicken breast meat containing white stripes during freezing for 12 mo. Samples of Pectoralis major muscle from male Cobb 500 broilers containing white stripes in moderate and severe degrees were used, as well as samples from a control group (normal – absence of myopathies). Part of the samples (n = 60; n = 20 for each severity degree) were analyzed on the day of collection (beginning) and the rest (n = 240) was frozen (−20°C) for up to 12 mo. At the end of each proposed freezing period (3, 6, 9, and 12 mo), color, pH, water holding capacity, cooking loss, tenderness, lipid oxidation, chemical composition, cholesterol and collagen concentrations, myofibrillary fragmentation, and sarcomere length were analyzed. Microbiological analysis of samples was performed at the beginning and after 12 mo of freezing. Myopathy linked to freezing showed results of increased meat tenderness, with reduction of crude protein and mineral matter and increase of moisture, fat, and cholesterol, without affecting the meat's collagen percentages. However, these variations with the onset of myopathy do not compromise the consumption of broiled chicken breast meat, kept frozen for up to 12 mo.

Key words: chicken breast meat, meat quality, storage, white striping

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INTRODUCTION

Brazil is the third largest world producer of chicken meat with over 13 million tons of meat produced in 2020. In addition, the country is the largest world exporter and provided around 4 million tons of the total produced in 2020. Of the total exported, 66.99% were sold in cuts, 25.26% as whole carcasses, 2.16% were destined for the production of processed products, 3.14% were sold in salted form and 2.45% in the form of sausages (ABPA, 2021).

Due to the high demand for chicken meat, the market is constantly under adaptation in order to achieve better productivity rates. Therefore, genetic improvement is the main tool to achieve such goal, since it is used to select strains with high growth potential (Kuttappan et al., 2013). However, advances in the growth and yield of chicken meat have resulted in muscle changes, turning pectoral myopathies into the focus of current researches. Thus, a relationship between the occurrence of pectoral myopathies and the increase in breast weight and bird's age is suggested (Mudalal et al., 2015). In this sense, the appearance of whitish striping marks at different degrees of severity has increased the need for studies on physical changes, chemical and sensory effects that genetic progress can impose to the quality of the meat produced. The lesions known as white stripes (Kuttappan et al., 2012a) act as potential disadvantages for consumer acceptance and purchase intention of poultry meat. White Striping myopathy is characterized by the appearance of white stripes on the breast surface, often accompanied by signs of hemorrhage, which can result in consumer rejection and, consequently, economic loss.

Meat is marketed in a frozen form with the aim of increasing the shelf life and maintaining the quality of the product (Demirok et al., 2013). Thus, Adabi and

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Soncu (2019) evaluated chicken breast meat affected by "White Striping" myopathy submitted to freezing temperature (−18°C) for 30 d. However, Brazilian industry recommends a maximum period of 1 yr for marketing frozen chicken meat at −18°C and there are few studies in the literature regarding possible changes in samples affected by myopathy during long-term storage under freezing. Given this scenario, this study proposes to evaluate possible changes in the quality of chicken breast meat affected by white striping during freezing for 12 mo.

MATERIALS AND METHODS

Place and Period of Execution

The present study was developed at the Laboratory of Food Analysis of Animal Origin (**LaOra**) of the Technology Department of the Faculty of Agricultural and Veterinary Sciences − FCAV/UNESP, Jaboticabal Campus, São Paulo, Brazil (21° 08′ S, 48° 11′ W, 583 m altitude).

Sample Collection and Experimental Procedure

Three hundred samples of breast meat from male Cobb 500 broilers reared in a traditional intensive system and slaughtered at 45 d of age were used. The samples were acquired in a commercial slaughterhouse inspected by the Federal Inspection Service (SIF, Brazil). Birds were slaughtered according to the slaughterhouse routine. Bone-free and skin-free samples were classified by trained people according to the methodology employed by Kuttappan *et al.* (2012b) in: moderate (n = 100) − presence of stripes less than 1-mm thick; severe (n = 100) − presence of stripes with thickness greater than 1 mm verified over the entire length of the breast fillet) and normal (n = 100) − absence of myopathies. The normal breast fillet samples were used as control group. After collection, samples were separated for analysis on the same day (beginning) and transported to the University's laboratory under refrigeration conditions (± 4°C) for further quality analysis.

The samples separated for freezing were identified, weighed individually and quickly frozen in a freezing tunnel (−40°C) and, subsequently, were stored in a freezer at −20°C ± 0.5 °C for 3, 6, 9, and 12 mo (the samples were placed in polyethylene bags with no vacuum, previously identified, and sealed with plastic coated wire tie). The physical-chemical analyses described below were performed on non-frozen samples (on the day of collection; n = 20 for each severity degree and control group) and after 3, 6, 9, and 12 mo of storage (n = 20 for each severity level and control group; total n = 60, for each period), being the samples for each period (3, 6, 9, and 12 mo) frozen in a freezer (−20°C). Freezing was carried out for up to 12 mo as it is the maximum period of shelf life determined by Brazilian legislation (MAPA, 1998).

Physical Analysis

Color The color (luminosity - L *; red intensity - a * and yellow intensity - b *) was determined using a Minolta CR-400 colorimeter (Konica Minolta Sensing, Inc., Osaka, Japan) (settings: diffused lighting / 0 viewing angle, illuminant D65, specular component included) calibrated to a white standard. The equipment was positioned in 3 different locations on the external surface of the *Pectoralis major* muscle (which was previously in contact with the skin) and also in 3 different areas on the muscle's inner surface (which was in contact with the sternum bone).

Afterward, the pitch angle (h *) was calculated using the formula below:

$$h* = \left(\arctan\left(\frac{b*}{a*}\right)\right) \times 57.3$$

Arctan is the tangent arc and 57.3 is the factor that converts the result of h * from radians to the unit in degrees (Ramos and Gomide, 2017).

pH, Water Holding Capacity, Cooking Weight Loss, and Shear Strength The meat's pH was evaluated in triplicate using a digital pH meter (Testo 205, Testo Inc., Sparta, NJ) equipped with a penetration electrode, which was inserted into the cranial part of each sample.

The water holding capacity (**WHC**) was determined in triplicate as described by Hamm (1960). The samples were cooked to evaluate cooking losses according to the method described by Honikel (1987), and the results were obtained by the difference between the initial and final weights, expressed as a percentage. The shear strength was evaluated using the Warner-Bratzler device coupled to the Texture Analyzer TA-XT2i texturometer. From each cooked sample, three subsamples with a section area of 1 cm^2 were obtained, which were placed with the fibers oriented perpendicularly to the Warner-Bratzler device and subjected to cut (Lyon *et al.*, 1998). The force required to shear the samples was expressed in Newton.

Thawing Weight Loss and Exudate Soluble Protein- Thawing weight loss was defined as the difference between the initial and final weights of each sample, before and after freezing, expressed as a percentage. The samples were thawed in vertical refrigerators (constant temperature of 4°C) for 12 h.

The protein soluble in the exudate was evaluated as described by Hartree (1972).

Chemical Analysis

Myofibrillary Fragmentation Index, Sarcomere Length, and Collagen Myofibrillary fragmentation index (MFI) was determined as described by Culler et al. (1978), using the biuret method (Gornall et al., 1949) to determine the concentration of proteins in the suspension of myofibrils. MFI was calculated according to the following formula: MFI = optical density × 200. The sarcomere length was determined as

described by Cross et al. (1981). From each defrosted sample (these being frozen post rigor mortis), we manually obtained 0.5 g subsamples from each thawed samples from the end of the muscle fragment, which were removed with a scalpel and placed in a 50 mL falcon tube, to which were added 15 mL of potassium iodide (13.28 g / 1L of distilled water) and 15 mL of potassium chloride (5.96 g/L of distilled water). Subsequently, samples were homogenized in an ultra-turrax at 15,000 G for 30 s. The slides were made with a drop of the homogenate on a microscope slide and immediately covered with a coverslip. The readings were performed under a phase-contrast microscope (Novel BM2100) at 1,000× magnification (100× objective, 10× eyepiece) after a drop of immersion oil was applied. The sarcomere length was expressed in micrometers.

For the quantification of concentrations of total, soluble and insoluble collagen, the technique for determining the hydroxyproline amino acid was used according to a methodology adapted by Carvalho et al. (2021). Five grams of raw frozen meat cut into small pieces were weighed in 50 mL falcon tubes. Twenty mL of distilled water were added and the sample was taken to water bath (80°C for 2 h). Afterwards, the samples were homogenized in ultra-turrax at a speed of 12,650 turns per 1 min (Marconi MA102, Marconi laboratory equipment Ltda., Piracicaba, São Paulo, Brazil) and centrifuged at a speed of 2,300 turns for 15 min (Himac CR22N, Hitachi Koki from Brazil Ltda., Indaiatuba, São Paulo, Brazil). After centrifugation, the samples were transferred to autoclavable tubes, in which the solid fraction separated from the liquid fraction. Thirty mL of 6N HCl was added to the liquid fraction and 50 mL of 6N HCl was added to the solid fraction. Both were taken to an autoclave (Phoenix Luferco AV-75 Plus, Phoenix Scientific Equipment Industry and Trade Ltda., Araraquara, São Paulo, Brazil) for 4 h under temperature of 120°C and pressure of 1 atm. After the hydrolysis in an autoclave, the pH of all samples were adjusted to 6.0 (with MS Tecnopon mPA-210, MS Tecnopon Special Equipment Ltda., Piracicaba, São Paulo, Brazil) using a 2N NaOH solution. The solid fraction was filtered in a 250 mL volumetric flask, the liquid fraction was filtered in a 100 mL volumetric flask and the volume of the flasks was filled with distilled water. Subsequently, a 10 mL aliquot was removed from the filtered samples and transferred to a new volumetric flask (100 mL for solid fraction and 50 mL for liquid fraction). Again, the volume of the flasks was filled with distilled water. Then, a 2 mL aliquot of the solid fraction and liquid fraction was transferred in duplicate to test tubes, to which 1 mL of oxidation reagent (Chloramine-T 1 at 41%) and 1 mL of color (10 g of p-dimethyl amino benzaldehyde in 35 mL of 60% perchloric acid and 65 mL of isopropanol) were added. At the end of this process, the samples were taken to water bath for a period of 15 minutes, previously heated to a temperature of 60°C, and the samples were read on a spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) with a wavelength of 560 nm. The results for soluble collagen concentration were obtained through the liquid fraction of the sample and the results for insoluble collagen concentration were obtained through the solid fraction. A standard curve was calculated using a solution with known hydroxyproline concentration. Calculations of collagen concentrations were performed according to the formulas, where F is equal to 8.33 (mean absorbance values equivalent to 1 mg of hydroxyproline obtained from the standard curve) and 7.14 is the conversion factor of hydroxyproline in collagen.

Chemical Composition and Lipid Oxidation The chemical composition was determined in natura and in samples frozen for 3, 6, 9, and 12 mo. The samples were lyophilized (Super Modulyo 220, Thermo Fisher Scientific Inc., Waltham, MA) and the means for later determination of protein and mineral matter concentrations preceded by AOAC (2011), methods 977.14 and 920.153, respectively. The percentage of moisture was determined by the difference between the weight of the samples before and after lipophilization (AOAC 2011; method 950.46).

Lipid oxidation was determined in fresh samples and in samples frozen for3 and 6, 9, and 12 mo by thiobarbituric acid reactive substances (**TBARs**) test, according to the methodology described by Vyncke (1970).

Minerals Initially, the nitric-perchloric digestion of the ashes that came out of the analysis of mineral matter was performed to obtain a mineral extract from the samples (AOAC, 1984). Subsequently, the atomic absorption spectrophotometry technique was used to assess the concentration of minerals (except phosphorus). For the phosphorus analysis, the molybidate-vanadate spectrophotometric method was used. Such method consists of using a molybidate solution, which reacts with the phosphorus of the mineral extract, previously prepared in the nitric-perchloric digestion. The mineral extract was subsequently analyzed by the technique of colorimetric spectrophotometry to measure the amount of phosphorus present in the sample (Silva and Queiroz, 2005).

Microbiological Evaluation The microbiological analyses of the samples in-natura and after 12 mo of freezing were carried out according to the protocols "Official analytical methods for microbiological analysis for the control of products of animal origin" adopted by the Ministry of Agriculture and Supply (BRASIL, 2003) and the results were compared with the standards acceptable by IN 62/2003 (BRASIL, 2003) for total and thermo tolerant coliforms at 45°C, coagulase positive Staphylococcus and *Salmonella* spp.

Statistical Analysis

The data obtained from the physicochemical analyses were analyzed through a completely randomized experimental design (**CRD**) in a 3 × 5 factorial scheme (3 myopathy severity degrees and 5 storage periods) with 20 replications. Results were analyzed through the General Linear Models procedure of the Statistical Analysis System (SAS Institute Inc., 2002−2003, Cary, NC). In

the case of microbiological analyses, DIC was used in a 3 × 2 factorial scheme (2 severity degrees of myopathy, plus the control group, and 2 storage periods) with 20 repetitions each. All data were tested by analysis of variance (**ANOVA**) and compared by Bonferroni test at a significance level of 5%.

RESULTS AND DISCUSSION

There was no significant interaction between severity degree and freezing period for the variables luminosity (L*), red intensity (a*), yellow intensity (b*), and pitch angle (h*) analyzed on the outer and inner surfaces of samples (Table 1).

The meat affected by the moderate and severe degrees of White Striping myopathy showed higher ($P < 0.0001$) luminosity (L*) and yellow intensity (b*) on the outer surface than samples classified as normal. The severe degree had a higher ($P < 0.0001$) value of L* and b* than the moderate degree of White Striping myopathy.

These results are explained by the degradation of myofibrillar proteins, which leads to a reduction in muscle protein, providing the increased concentration of free water, resulting in increased luminosity and red intensity (Petracci et al., 2014; Soglia et al., 2016). Regarding the accumulation of water present on the surface, there was greater reflection of the light rays, increasing the values of L* in samples affected by White Striping. Recent studies (Baldi et al., 2018; Baldi et al., 2019; Mello et al., 2021) have reported changes in breast meat color in broilers affected by myopathies according to the sampled region (surface layers or deep layers).

Regardless of the involvement by myopathy, the values of L* and a* on the outer and inner surfaces decreased ($P < 0.05$) and the values of b* and h* increased ($P < 0.05$) after 12 mo of freezing. This probably occurred due to the protein degradation that provides the appearance of fibrosis and interstitial inflammation (Kuttappan et al., 2013; Petracci et al., 2013). The reduction in the value of L* after 12 mo of freezing was due to the denaturation of proteins during defrosting (Olivo and Olivo, 2005); a fact that may have occurred in the research by Soglia et al. (2019) who observed an overall reduction in L* after freezing and subsequent thawing of the meat affected by White Striping myopathy. This result might be ascribed to the alteration in meat structure resulting from the formation of ice crystals during freezing that might predominantly affect the structure of the White Striping muscles (**WS**). In detail, disrupting the muscle cells, ice crystals likely aggravated the profound histological lesions previously observed in WS (Soglia et al., 2016) thus resulting in a further altered muscle architecture leading to a different scattering of the incident light.

The loss of myoglobin (the pigment responsible for the red coloration of the meat) through the meat exudate may have caused a reduction in the red intensity of all

Table 1. Mean values of luminosity (L*), red intensity (a*), yellow intensity (b*) and the angle of hue (h*) of the breast meat of broilers affected by frozen White Striping myopathy for up to 12 mo.

Factors	L*	a*	b*	h* (degrees)
		Outer surface		
		Severity degree (SD)		
Normal	58.18 ± 3.01^{C}	1.73 ± 0.98	4.49 ± 2.01^{C}	73.66 ± 9.74
Moderate	59.26 ± 3.16^{B}	1.71 ± 0.91	5.51 ± 1.97^{B}	73.02 ± 9.44
Severe	60.78 ± 3.71^{A}	1.69 ± 0.99	6.38 ± 2.23^{A}	72.98 ± 10.01
		Storage time (ST)		
Beginning	63.46 ± 2.68^{A}	2.01 ± 0.99^{A}	4.34 ± 1.84^{E}	69.98 ± 9.25^{C}
3 mo	61.48 ± 2.14^{B}	1.83 ± 0.87^{B}	5.24 ± 1.93^{D}	69.30 ± 8.93^{C}
6 mo	58.88 ± 2.89^{C}	1.69 ± 0.93^{C}	5.87 ± 2.21^{C}	74.79 ± 9.93^{B}
9 mo	57.50 ± 2.57^{D}	1.56 ± 0.94^{D}	6.29 ± 1.98^{B}	74.76 ± 8.55^{B}
12 mo	56.68 ± 2.87^{E}	1.40 ± 0.86^{E}	6.86 ± 2.02^{A}	76.27 ± 8.81^{A}
		P-value		
P (DS)	<0.0001	0.4929	<0.0001	0.7160
P (ST)	0.0007	<0.0001	<0.0001	0.0050
P Int (DS × ST)	0.7007	0.5629	0.5067	0.1884
		Inner surface		
		Severity degree (SD)		
Normal	59.24 ± 2.96	1.60 ± 0.77	8.40 ± 1.91	78.94 ± 5.78
Moderate	59.60 ± 3.16	1.61 ± 0.81	8.80 ± 1.92	79.49 ± 5.68
Severe	59.41 ± 3.02	1.65 ± 0.71	8.98 ± 2.04	80.83 ± 5.32
		Storage time (ST)		
Beginning	61.63 ± 3.44^{A}	1.96 ± 0.82^{A}	6.19 ± 1.37^{E}	79.83 ± 6.29^{B}
3 mo	61.12 ± 2.95^{A}	1.68 ± 0.65^{B}	7.08 ± 1.63^{D}	80.02 ± 5.11^{B}
6 mo	60.11 ± 2.79^{A}	1.43 ± 0.70^{C}	8.51 ± 1.69^{C}	80.32 ± 6.57^{A}
9 mo	57.72 ± 2.97^{B}	1.22 ± 0.88^{D}	9.02 ± 1.55^{B}	80.75 ± 5.75^{A}
12 mo	56.52 ± 3.37^{B}	1.18 ± 0.75^{E}	9.56 ± 1.45^{A}	80.84 ± 5.31^{A}
		P-value		
P (DS)	0.7609	0.3571	0.3081	0.1027
P (ST)	<0.0001	<0.0001	<0.0001	<0.0001
P Int (DS × ST)	0.4572	0.2938	0.2864	0.0988

$^{A-E}$Means followed by different letters (in the columns) differ from each other by the Bonferroni test ($P < 0.05$).

samples during the 12 mo of freezing. Nonetheless, oxidative reactions can occur during the frozen storage of meat and have proteins and lipids as the main target (Soglia et al., 2019). The storage caused loss of exudate by protein denaturation, generating an increase in the yellow intensity over the 12 mo of freezing. In detail, primary lipid oxidation (peroxidation) might be initiated during frozen storage of the meat leading to radical secondary lipid oxidation upon thawing (Leygonie et al., 2012a), a fact that increase the b*. The increase in the h* value over 12 mo of freezing may have occurred due to the loss of exudate and hemoglobin, thus providing the concentration of lipids in the meat muscle, causing an increase in yellow intensity and a reduction in red intensity. In addition, with the loss of exudate, there is an increase in the concentration of lipids (since these are not water-soluble), which results in an increase in b* and, consequently, a reduction in the value of a*.

There was no significant interaction between severity degree and freezing period for the pH variable (Table 2).

The meat affected by the moderate and severe degrees of White Striping myopathy showed higher ($P < 0.0001$) pH values than samples classified as normal. The severe degree had a higher ($P < 0.0001$) pH value than the moderate degree of White Striping myopathy (Table 2). Most of the recently published researches on White Striping myopathy in chicken breast describe a pH increase and higher incidences in heavy birds (Kuttappan et al., 2017; Baldi et al., 2018, 2019; Gratta et al., 2019). The reduced muscle vascularization and glycogen reserves observed in heavy chickens may compromise the energy supply to the muscle fibers (Alnahhas et al., 2016; Baldi et al., 2018). The higher pH values could then be explained by the correlation between glycogen storage and breast muscle weight (Le Bihan-Duval et al., 2008), since larger fillets may present reduced glycolytic potential, which would result in a higher pH (Baldi et al., 2019). There was a reduction ($P < 0.0001$) in the pH after 12 mo of freezing. According to Sylvestre et al. (2001), during the freezing of the meat, reactions linked to protein denaturation may occur, which produce peptides and free amino acids, resulting in accumulation of metabolites influencing the final pH value.

There was an interaction ($P < 0.05$) between the factors for the variables water holding capacity, cooking weight loss and shear strength (Table 3). The meat affected by the moderate and severe degrees of White Striping myopathy showed higher WHC than samples classified as normal. The severe degree had a higher WHC than the moderate grade degree of White Striping myopathy. This result is associated with that of high pH in samples affected by white striping myopathy. The myofibrillar proteins have a more direct structural role in determining meat quality traits. The increase in water-holding capacity may result from increased pH and enzymatic degradation of myofibrillary structure (Pearce et al., 2011; Li et al., 2014). According to Ramos and Gomide (2017), when the pH value increases, the distance between the myosin filaments increases due to enzymatic degradation of myofibrillary structure (Pearce et al., 2011; Li et al., 2014) causing to change in the isoelectric point of myofibrillar proteins (Walter, 1975), thus promoting water holding associated with myofibrillar proteins, providing an increase in WHC. Conversely, during the 12 mo of freezing, there was denaturation of sarcoplasmic proteins and a reduction in pH, caused by the long freezing time that causes the formation of large ice crystals in the extracellular medium decreasing myofilament spacing and promoting water loss by exudation increasing free water concentration, causing the water holding capacity to decrease (Leygonie et al., 2012). Indeed, by leading to the disruption of the cellular organelles and ultimately exposing the myofibrillar proteins to oxidative enzymes and prooxidant molecules, freezing results in an increased protein oxidation (Xia et al., 2009; Soglia et al., 2019), causing the water holding capacity to decrease.

The meat affected by the moderate and severe degrees of White Striping myopathy showed less cooking weight loss (**CWL**) than samples classified as normal. The severe degree had lower CWL than the moderate degree of

Table 2. Mean pH values, water holding capacity (WHC), cooking weight loss (CWL), and shear strength of broiler chicken breast affected by frozen white striping myopathy for up to 12 mo.

Factors	pH	WHC (%)	CWL (%)	FC (N)
		Severity degree (SD)		
Normal	5.91 ± 0.14^{C}	60.39 ± 23.84^{C}	29.59 ± 3.33^{A}	33.45 ± 7.90^{A}
Moderate	5.96 ± 0.16^{B}	65.48 ± 25.87^{B}	26.88 ± 2.97^{B}	27.86 ± 8.99^{B}
Severe	6.00 ± 0.17^{A}	70.25 ± 27.83^{A}	23.87 ± 3.21^{C}	20.45 ± 6.52^{C}
		Storage time (ST)		
Beginning	6.14 ± 0.09^{A}	77.84 ± 4.59^{A}	23.38 ± 3.54^{E}	35.19 ± 8.98^{A}
3 mo	6.01 ± 0.12^{B}	75.71 ± 3.71^{B}	25.44 ± 3.92^{D}	28.53 ± 6.52^{B}
6 mo	5.93 ± 0.13^{C}	73.12 ± 4.18^{C}	26.61 ± 3.14^{C}	25.32 ± 12.58^{C}
9 mo	5.85 ± 0.10^{D}	71.07 ± 4.09^{D}	28.47 ± 3.11^{B}	22.52 ± 11.39^{D}
12 mo	5.80 ± 0.11^{E}	68.03 ± 3.65^{E}	29.81 ± 3.09^{A}	17.91 ± 5.62^{E}
		P-value		
P (SD)	<0.0001	<0.0001	<0.0001	<0.0001
P (ST)	<0.0001	<0.0001	<0.0001	<0.0001
P Int (SD × ST)	0.6816	<0.0001	<0.0001	<0.0001

[A-E]Means followed by different letters (in the columns) differ from each other by the Bonferroni test ($P < 0.05$).

Table 3. Mean pH values, water holding capacity (WHC), cooking weight loss (CWL), and shear strength of broiler chicken breast affected by frozen white striping myopathy for up to 12 mo.

WHC (%)					
	DS (n = 20)				
ST (n = 20)	Normal	Moderate	Severe		*P*-value
Beginning	75.03 ± 3.31^{Ac}	78.71 ± 3.64^{Ab}	80.05 ± 3.40^{Aa}		
3 mo	74.57 ± 3.80^{Bc}	76.07 ± 4.04^{Bb}	78.73 ± 4.18^{Ba}	(SD)	<0.0001
6 mo	72.39 ± 3.77^{Cc}	74.28 ± 3.48^{Cb}	76.12 ± 3.05^{Ca}	(ST)	<0.0001
9 mo	70.67 ± 3.01^{Dc}	71.91 ± 3.51^{Db}	74.94 ± 3.14^{Da}	(SD x ST)	<0.0001
12 mo	66.98 ± 3.53^{Ec}	68.36 ± 3.78^{Eb}	69.31 ± 3.90^{Ea}		
CWL (%)					
	Normal	Moderate	Severe		*P*-value
Beginning	24.74 ± 2.78^{Ea}	23.51 ± 2.68^{Eb}	21.88 ± 2.35^{Ec}		
3 mo	27.17 ± 1.96^{Da}	26.17 ± 1.95^{Db}	22.98 ± 2.46^{Dc}	(SD)	<0.0001
6 mo	28.47 ± 2.65^{Ca}	27.15 ± 3.08^{Cb}	24.22 ± 2.99^{Cc}	(ST)	<0.0001
9 mo	30.24 ± 2.35^{Ba}	29.36 ± 2.34^{Bb}	25.81 ± 2.28^{Bc}	(SD × ST)	<0.0001
12 mo	32.58 ± 2.28^{Aa}	30.63 ± 2.26^{Ab}	26.20 ± 2.45^{Ac}		
FC (N)					
	Normal	Moderate	Severe		*P*-value
Beginning	40.28 ± 8.14^{Aa}	37.98 ± 8.21^{Ab}	27.32 ± 7.01^{Ac}		
3 mon	39.85 ± 7.74^{Ba}	26.14 ± 7.05^{Bb}	19.62 ± 7.30^{Bc}	(SD)	<0.0001
6 mo	35.65 ± 9.11^{Ca}	21.03 ± 8.99^{Cb}	19.27 ± 8.29^{Cc}	(ST)	<0.0001
9 mo	28.57 ± 7.94^{Ca}	20.96 ± 7.82^{Cb}	18.04 ± 8.08^{Cc}	(SD × ST)	<0.0001
12 mo	22.69 ± 8.80^{Da}	16.42 ± 8.97^{Db}	14.63 ± 9.01^{Dc}		

Means followed by different letters in the columns (upper case) and in the lines (lower case) are significantly different by the Bonferroni test ($P < 0.05$). Abbreviations: SD, severity degree; ST , storage time; SD × ST, interaction.

White Striping myopathy. Previous research (Mudalal et al., 2015; Trocino et al., 2015; Tasoniero et al., 2016; Zambonelli et al., 2016) with agreeing results for cooking loss indicates that more fluid is lost in samples affected by myopathies when compared to meat classified as normal. This is especially true if more than one myopathy is observed in the sample (Gratta et al., 2019). However, in the present study, cooking loss was found to decrease with the severity degree. The increased cooking loss reported by the above mentioned researchers was thought to be related to a reduction of myofibrillar proteins, fiber shortening and increased connective tissue or collagen contents, which might reduce water-holding capacity and, consequently, increase cooking loss. In our study, the data found of WHC and CWL may have been due to the influence of the pH of the meat.

Over time, CWL increased ($P < 0.05$), both for normal samples and for those affected by White Striping myopathy (Table 3). This increase is related to the denaturation of sarcoplasmic proteins (Shenouda, 1980; Estevez et al., 2011) in protein oxidation caused by the exposure of myofibrillar proteins (Xia et al., 2009; Soglia et al., 2019) and also by the decrease in pH over the storage time of these meats ($P < 0.05$), increasing free water concentration, resulting in the production of a greater amount of exudate out of the meat during cooking. However, samples affected by White Striping myopathy showed less shear strength when compared to normal samples. Such tenderness may be related to the increase in fat and the lower integrity of muscle fibers that is provided by the myopathy onset (Tasoniero et al., 2016). During the freezing process, there was a reduction in shear strength and, consequently, an increase in tenderness in all samples. This result occurred due to the proteolysis of muscle fibers caused by the freezing process (Estevez et al., 2011).

There was no significant interaction between severity degree and freezing period for the variable protein, soluble in exudate (SP) (Table 4). The meat affected by the moderate and severe degrees of White Striping myopathy showed a higher ($P < 0.0001$) concentration of soluble protein in the exudate (SP) than samples classified as normal. The severe degree had a higher SP than the moderate degree of White Striping myopathy. Such result possibly occurred due to protein degradation, characteristic of myopathy (Kuttappan et al., 2013), because the more muscle protein is degraded, the greater the amount of free protein solubilized with the water present in the meat, which is exudated afterward (Table 4). Over 12 mo of freezing, there is an increase ($P < 0.0001$) in the concentration of soluble protein in the exudate, regardless of the involvement of myopathy, due to the denaturation of sarcoplasmic proteins by freezing (Shenouda, 1980; Estevez et al., 2011) and to the disruption of the cellular organelles causing the exposure of the myofibrillar proteins (Xia et al., 2009; Soglia et al., 2019), thus providing a greater amount of free protein that solubilizes with the water that is released from the meat during thawing.

There was an interaction ($P < 0.05$) between the myopathy severity degree and the storage time for the

Table 4. Mean values of soluble protein in exudate (SP) and weight loss on defrosting (WLD) from broilers affected by White Striping myopathy frozen for up to 12 mo.

Factors	SP (mg / mL drip)	WLD (g)
	Severity degree (SD)	
Normal	0.08 ± 0.01^{C}	16.30 ± 4.06^{A}
Moderate	0.09 ± 0.01^{B}	14.76 ± 2.47^{B}
Severe	0.10 ± 0.01^{A}	13.96 ± 3.21^{C}
	Storage time (ST)	
Beginning	-	-
3 mo	0.08 ± 0.005^{D}	12.15 ± 2.57^{D}
6 mo	0.09 ± 0.006^{C}	13.39 ± 2.06^{C}
9 mo	0.10 ± 0.007^{B}	16.42 ± 2.74^{B}
12 mo	0.11 ± 0.006^{A}	18.07 ± 1.71^{A}
	P-value	
P (SD)	<0.0001	<0.0001
P (ST)	<0.0001	<0.0001
P Int (SD × ST)	0.4001	<0.0001

	WLD (g)				
	SD (n = 20)				
ST (n = 20)	Normal	Moderate	Severe	*P*-value	
3 mo	14.06 ± 1.89^{Da}	11.54 ± 2.27^{Db}	10.84 ± 1.97^{Dc}		
6 mo	14.92 ± 1.86^{Ca}	13.11 ± 2.26^{Cb}	12.14 ± 1.91^{Cc}	(SD)	<0.0001
9 mo	17.19 ± 2.51^{Ba}	16.35 ± 2.51^{Bb}	15.73 ± 2.05^{Bc}	(ST)	<0.0001
12 mo	19.03 ± 2.01^{Aa}	18.05 ± 1.91^{Ab}	17.15 ± 2.04^{Ac}	(SD × ST)	<0.0001

[A-E]Means followed by different letters (in the columns) differ from each other by the Bonferoni test ($P < 0.05$).

variables thawing weight loss (**TWL**) and exudate volume (Table 5). The increase in the myopathy severity provided less ($P < 0.05$) thawing weight loss in all storage periods due to the higher pH in samples affected by White Striping myopathy, which provided the approximation of myosin filaments, resulting in increased water holding, causing a reduction in the weight loss of meat during thawing (Ramos and Gomide, 2017). Over 12 mo, there was an increase in weight loss in all samples, because during freezing, the protein compounds lose water which turns into ice crystals. Thus, there is appearance of aggregates of myosin and actin, promoting the denaturation of the tertiary and secondary structures of sarcoplasmic proteins. With denaturation, water is released to the external environment of the meat (Shenouda, 1980).

There was less ($P < 0.05$) exudate production in samples affected by White Striping myopathy than in normal samples. The severe degree produced less exudate compared to the moderate myopathy degree. This result is associated with the variation found in the meat's pH, which influenced the distance between the myosin filaments (Ramos and Gomide, 2017) providing a reduction in the outflow of liquids with water-soluble substances. Over 12 mo of storage, there was an increase ($P < 0.05$) in the amount of exudate produced after thawing all samples. This is due to the denaturation of sarcoplasmic proteins (Shenouda, 1980; Estevez et al., 2011) and the protein oxidation caused by the exposure of myofibrillar proteins (Xia et al., 2009; Soglia et al., 2019), so the longer the meat remains frozen, the greater the protein denaturation, increasing the amount of water that is lost during the meat thawing.

There was significant interaction between the myopathy severity degree and the storage time for the variables myofibrillar fragmentation index, sarcomere length and

Table 5. Mean values and standard deviation of the myofibrillary fragmentation index (MFI), sarcomere length, soluble, insoluble collagen, and total breast of broilers affected by White Striping myopathy frozen for 12 mo.

Factors	MFI	Sarcomere length (μm)	Soluble collagen (%)	Insoluble collagen (%)	Total collagen (%)
			Severity degree (SD)		
Normal	79.22 ± 13.56^{C}	1.63 ± 0.09^{C}	0.13 ± 0.06^{A}	0.14 ± 0.07	0.30 ± 0.11
Moderate	87.72 ± 14.71^{B}	$1.68 \pm 0,07^{B}$	0.13 ± 0.07^{A}	0.14 ± 0.07	0.29 ± 0.10
Severe	106.81 ± 11.40^{A}	$1.72 \pm 0,08^{A}$	0.11 ± 0.05^{B}	0.14 ± 0.06	0.30 ± 0.12
			Storage time (ST)		
Beginning	78.71 ± 15.28^{E}	1.54 ± 0.12^{D}	0.22 ± 0.06^{A}	0.20 ± 0.05^{A}	0.48 ± 0.18^{A}
3 mo	83.52 ± 18.87^{D}	1.67 ± 0.08^{C}	0.17 ± 0.04^{B}	0.19 ± 0.06^{A}	0.37 ± 0.16^{B}
6 mo	95.73 ± 13.98^{C}	1.69 ± 0.06^{B}	0.13 ± 0.07^{C}	0.15 ± 0.04^{B}	0.31 ± 0.14^{C}
9 mo	100.07 ± 20.15^{B}	1.74 ± 0.07^{A}	0.10 ± 0.09^{D}	0.10 ± 0.05^{C}	0.25 ± 0.11^{D}
12 mo	110.15 ± 17.84^{A}	1.74 ± 0.06^{A}	0.04 ± 0.09^{E}	0.05 ± 0.06^{D}	0.09 ± 0.13^{E}
			P-value		
P (SD)	<0.0001	<0.0001	<0.0001	0.8249	0.1169
P (ST)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P Int (SD × ST)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[A-E]Means followed by different letters (in the columns) differ from each other by the Bonferroni test ($P < 0.05$).

Table 6. Mean values and standard deviation of the myofibrillary fragmentation index (MFI), sarcomere length, soluble, insoluble collagen and total breast of broilers affected by White Striping myopathy frozen for 12 mo.

MFI					
			SD (n = 20)		
ST (n = 20)	Normal	Moderate	Severe	*P*-value	
Beginning	63.29 ± 5.87^{Ec}	78.06 ± 7.05^{Eb}	88.73 ± 6.25^{Ea}		
3 mo	69.36 ± 5.56^{Dc}	80.77 ± 5.82^{Db}	106.51 ± 7.38^{Da}	(SD)	<0.0001
6 mo	74.27 ± 7.38^{Cc}	95.47 ± 6.88^{Cb}	111.69 ± 7.01^{Ca}	(ST)	<0.0001
9 mo	80.05 ± 6.56^{Bc}	99.67 ± 6.15^{Bb}	120.50 ± 6.97^{Ba}	(SD × ST)	<0.0001
12 mo	93.73 ± 7.74^{Ac}	110.06 ± 7.25^{Ab}	126.65 ± 6.99^{Aa}		
Sarcomere length (μm)					
			SD (n = 20)		
ST (n = 20)	Normal	Moderate	Severe	*P*-value	
Beginning	1.43 ± 0.05^{Dc}	1.54 ± 0.02^{Db}	1.65 ± 0.07^{Da}		
3 mo	1.65 ± 0.05^{Cc}	1.66 ± 0.05^{Cb}	1.70 ± 0.07^{Ca}	(SD)	<0.0001
6 mo	1.67 ± 0.05^{Bc}	1.69 ± 0.05^{Bb}	1.73 ± 0.05^{Ba}	(ST)	<0.0001
9 mo	1.70 ± 0.04^{Ac}	1.75 ± 0.05^{Ab}	1.76 ± 0.04^{Aa}	(SD × ST)	<0.0001
12 mo	1.70 ± 0.03^{Ac}	1.75 ± 0.04^{Ab}	1.77 ± 0.06^{Aa}		
Soluble collagen (%)					
			SD (n = 20)		
ST (n = 20)	Normal	Moderate	Severe	*P*-value	
Beginning	0.21 ± 0.019^{Aa}	0.22 ± 0.016^{Aa}	0.23 ± 0.012^{Aa}		
3 mo	0.17 ± 0.017^{Ba}	0.16 ± 0.013^{Ba}	0.17 ± 0.020^{Ba}	(SD)	<0.0001
6 mo	0.14 ± 0.017^{Ca}	0.13 ± 0.019^{Ca}	0.12 ± 0.017^{Ca}	(ST)	<0.0001
9 mo	0.09 ± 0.018^{Da}	0.10 ± 0.20^{Da}	0.10 ± 0.018^{Da}	(SD × ST)	<0.0001
12 mo	0.04 ± 0.015^{Ea}	0.03 ± 0.18^{Ea}	0.04 ± 0.016^{Ea}		
Insoluble collagen (%)					
			SD (n = 20)		
ST (n = 20)	Normal	Moderate	Severe	*P*-value	
Beginning	0.20 ± 0.02^{Aa}	0.21 ± 0.04^{Aa}	0.20 ± 0.02^{Aa}		
3 mo	0.19 ± 0.03^{Aa}	0.20 ± 0.01^{Aa}	0.19 ± 0.05^{Aa}	(SD)	0.8249
6 mo	0.16 ± 0.05^{Ba}	0.15 ± 0.04^{Ba}	0.14 ± 0.03^{Ba}	(ST)	<0.0001
9 mo	0.09 ± 0.04^{Ca}	0.10 ± 0.02^{Ca}	0.10 ± 0.05^{Ca}	(SD × ST)	<0.0001
12 mo	0.06 ± 0.02^{Da}	0.04 ± 0.03^{Da}	0.05 ± 0.04^{Da}		
Total collagen (%)					
			SD (n = 20)		
ST (n = 20)	Normal	Moderate	Severe	*P*-value	
Beginning	0.48 ± 0.08^{Aa}	0.48 ± 0.07^{Aa}	0.47 ± 0.05^{Aa}		
3 mo	0.37 ± 0.09^{Ba}	0.36 ± 0.09^{Ba}	0.38 ± 0.07^{Ba}	(SD)	0.1169
6 mo	0.32 ± 0.05^{Ca}	0.30 ± 0.07^{Ca}	0.31 ± 0.08^{Ca}	(ST)	<0.0001
9 mo	0.24 ± 0.08^{Da}	0.25 ± 0.06^{Da}	0.26 ± 0.09^{Da}	(SD × ST)	<0.0001
12 mo	0.10 ± 0.07^{Ea}	0.08 ± 0.06^{Ea}	0.09 ± 0.07^{Ea}		

Means followed by different letters in the columns (upper case) and in the lines (lower case) are significantly different by the Bonferroni test ($P < 0.05$). Abbreviations: SD, severity degree; ST, storage time; SD × ST, interaction.

for the concentrations of soluble, insoluble and total collagen (Table 6). The meat affected by the moderate and severe degrees of White Striping myopathy showed higher ($P < 0.05$) MFI than samples classified as normal at all storage periods. The severe degree had a higher MFI than the moderate degree of White Striping myopathy. Frozen storage over 12 mo provided an increase ($P < 0.05$) of the MFI in all samples. These results demonstrate that such muscular alteration promotes greater tenderness to the meat due to the integrity reduction of the muscular fibers and to the greater concentration of lipids, providing less resistance of the muscular fiber to shear (Tasoniero et al., 2016). MFI increased during freezing due to the degradation of sarcoplasmic proteins (Shenouda, 1980; Estevez et al., 2011) and the protein oxidation caused by the exposure of myofibrillar proteins (Xia et al., 2009; Soglia et al., 2019), making meat more tender over the time of freezing.

Regardless of the freezing time, samples affected by the myopathy severe degree showed a greater ($P < 0.05$) sarcomere length than samples of moderate degree and normal samples (Table 5). This result may have occurred due to the presence of muscle degeneration, changes in fiber size, flocular and vacuolar degenerations, infiltration of mononuclear cells, moderate mineralization, lipidosis, and interstitial inflammation that have been reported in previous research (Kuttappan et al., 2013; Petracci et al., 2013) which provided the hypertrophy of muscle fibers,

Table 7. Mean values of moisture, crude protein, mineral matter, and lipid oxidation (TBARS) of breast meat from broilers affected by White Striping myopathy frozen for up to 12 mo.

Factors	Moisture (%)	Protein (%)	Mineral matter (%)	TBARS (mg MDA / kg)
		Severity degree (SD)		
Normal	69.59 ± 1.84^{C}	23.23 ± 2.91^{A}	1.88 ± 0.41^{A}	0.566 ± 0.248^{C}
Moderate	70.69 ± 1.85^{B}	21.41 ± 2.44^{B}	1.75 ± 0.36^{B}	0.642 ± 0.293^{B}
Severe	71.63 ± 2.22^{A}	19.11 ± 3.14^{C}	1.60 ± 0.38^{C}	0.710 ± 0.250^{A}
		Storage time (ST)		
Beginning	72.93 ± 1.78^{A}	24.59 ± 2.04^{A}	2.28 ± 0.36^{A}	0.370 ± 0.108^{E}
3 mo	71.55 ± 1.14^{B}	22.66 ± 2.61^{B}	1.97 ± 0.45^{B}	0.493 ± 0.106^{D}
6 mo	70.47 ± 1.34^{C}	21.43 ± 2.63^{C}	1.65 ± 0.39^{C}	0.670 ± 0.070^{C}
9 mo	69.81 ± 1.54^{D}	19.47 ± 2.53^{D}	1.53 ± 0.41^{D}	0.793 ± 0.062^{B}
12 mo	68.41 ± 1.66^{E}	18.11 ± 2.26^{E}	1.32 ± 0.40^{E}	0.871 ± 0.123^{A}
		P-value		
P (SD)	<0.0001	<0.0001	<0.0001	0.8249
P (ST)	<0.0001	<0.0001	<0.0001	<0.0001
P Int (SD × ST)	<0.0001	<0.0001	<0.0001	<0.0001

[A-E]Means followed by different letters (in the columns) differ from each other by the Bonferroni test ($P < 0.05$).

interfering with the sarcomere length. An increase ($P < 0.05$) in the sarcomere length was observed in all samples studied during the 12 mo of freezing, which may probably have occurred due to the ice crystals that form during freezing and the protein degradation that occurs during freezing (Souza, 2006), as the volume of water expands when it changes from a liquid state to a case, the water that is inside the sarcomere causes its length to increase when it expands due to freezing (Ertbjerg and Puolanne, 2017). These results suggest meat tenderization during storage under freezing, and, according to Ramos and Gomide (2017), increase in the sarcomere length leads to an increase in meat tenderness. The results of the present research demonstrate the direct relationship between MFI and sarcomere length (Table 6), with the tenderness of the meat (Table 2). This relationship was found by Oliveira et al. (2021) in meat affected by wooden breast myopathy (**WBM**), who believes there is a direct relationship between sarcomere length and meat tenderness in which the longer the sarcomere length, the greater the meat tenderness. The same authors suggest the use of this relationship in chicken fillets affected by WBM. Koohmaraie et al. (1990) state there is a direct relationship between MFI and tenderness, with higher values of MFI correlating with lower shear strength and greater tenderness.

There was no effect ($P > 0.05$) of White Striping myopathy on the concentrations of soluble, insoluble, and total collagen in all storage periods (Table 6), suggesting there is no presence of fibrosis (replacement of muscle tissue by connective tissue). There was a reduction ($P < 0.05$) in the concentrations of soluble, insoluble, and total collagen with the freezing time in all samples, probably due to the degradation of hydroxyproline and proline in less complex substances, by the proteolytic action of cathepsins that break down collagen into water-soluble substances that are carried without exudate produced during thawing (Oliveira *et al.*, 1998).

There was an interaction ($P < 0.05$) between the severity degree of White Striping myopathy and the freezing period for the amount of moisture, protein, mineral matter and lipid oxidation (Table 7).

Samples of chicken muscle with moderate and severe degrees of White Striping myopathy showed higher concentrations of moisture and lower protein and mineral matter than samples of normal chicken. There was a reduction in the amount of moisture, protein and mineral matter in all samples over 12 mo of freezing.

The higher percentage of moisture in samples with myopathy is possibly explained by the high pH and water holding capacity (Tables 2 and 3) that resulted in less exudative meat. The increasing production of exudate after thawing the samples resulted in a reduction in moisture concentration over the 12 mo of storage. The lower protein concentration presented in samples containing stripes is due to the myodegeneration that occurs in the muscle with the onset of myopathy (Tasoniero et al., 2016). The reduction in protein content over the 12 mo of freezing is possibly due to the degradation of proteins (Shenouda, 1980; Estevez et al., 2011) which transform into less complex substances and are eliminated with the exudated liquid during thawing. The results of mineral matter can be explained by the percentage of moisture in the samples, as the samples classified as severe degree had higher moisture content, resulting in the dilution of the minerals.

Meat affected by the moderate and severe degrees of White Striping myopathy showed higher ($P < 0.05$) lipid oxidations (TBARS) than samples classified as normal at all storage periods. The severe degree had higher TBARS than the moderate degree of White Striping myopathy. Frozen storage over 12 mo provided an increase ($P < 0.05$) in TBARS in all samples. This variation is due to the greater amount of fat present in the samples affected by the myopathy, which results in greater lipid oxidation. According to Torres et al. (1989), the range of values from 0.6 to 2.0 mg of MDA/Kg provides the perception of rancidity in meat. This range of values is only verified after 6 mo of freezing for all treatments, however, the highest value was 0.96 mg MDA/Kg of sample, which is less than the critical value of 2.0 mg, showing we must be careful when using these meats for consumption and marketing after 6 mo of freezing.

There was no interaction ($P > 0.05$) between the severity degree of White Striping myopathy and the

Table 8. Mean values of calcium, magnesium, sodium, chlorine, sulfur, phosphorus, and potassium concentrations in broiler chicken affected by White Striping myopathy frozen for up to 12 mo.

Factors	Calcium (%)	Magnesium (%)	Sodium (%)	Chlorin (%)	Sulfur (%)	Phosphorus (g/Kg)	Potassium (g/Kg)
			Severity degree (SD)				
Normal	0.126 ± 0.045^{A}	0.89 ± 0.05	2.07 ± 0.13^{B}	0.02 ± 0.0	9.18 ± 0.17	9.46 ± 0.35^{A}	11.46 ± 0.36^{A}
Moderate	0.120 ± 0.043^{B}	0.89 ± 0.03	2.19 ± 0.15^{B}	0.02 ± 0.0	9.23 ± 0.22	9.64 ± 0.23^{A}	11.15 ± 0.46^{B}
Severe	0.100 ± 0.041^{C}	0.88 ± 0.04	2.49 ± 0.16^{A}	0.02 ± 0.0	9.19 ± 0.26	9.26 ± 0.25^{B}	10.97 ± 0.43^{C}
			Storage time (ST)				
Beginning	0.155 ± 0.052^{A}	0.92 ± 0.04^{A}	2.32 ± 0.24^{A}	0.02 ± 0.0	9.25 ± 0.17	9.84 ± 0.34^{A}	12.04 ± 0.52^{A}
3 mo	0.111 ± 0.047^{B}	0.89 ± 0.03^{B}	2.31 ± 0.21^{A}	0.02 ± 0.0	9.27 ± 0.19	$9.49 \pm 0,23^{B}$	11.20 ± 0.69^{B}
6 mo	0.112 ± 0.033^{B}	0.88 ± 0.04^{B}	2.27 ± 0.19^{B}	0.02 ± 0.0	9.29 ± 0.15	$9.19 \pm 0,28^{C}$	11.20 ± 0.49^{B}
9 mo	0.102 ± 0.050^{C}	0.87 ± 0.04^{B}	2.24 ± 0.23^{B}	0.02 ± 0.0	9.25 ± 0.17	9.38 ± 0.29^{B}	10.88 ± 0.70^{C}
12 mo	0.101 ± 0.033^{C}	0.86 ± 0.05^{C}	2.11 ± 0.26^{C}	0.02 ± 0.0	9.27 ± 0.16	9.38 ± 0.38^{B}	10.66 ± 0.54^{D}
			P-value				
P (SD)	0.0035	0.1137	0.0001	0.2121	0.9910	<0.0001	<0.0001
P (ST)	0.0013	0.0452	0.0298	0.3837	0.295	<0.0001	<0.0001
P Int (SD × ST)	0.3422	0.1233	0.4754	0.1922	0.1912	<0.0001	<0.0001

[A-E]Means followed by different letters (in the columns) differ from each other by the Bonferroni test ($P < 0.05$).

freezing period for the amount of calcium, magnesium, sodium, chlorine, and sulfur (Table 8).

The meat affected by the moderate and severe degrees of White Striping myopathy showed higher ($P = 0.0035$) calcium concentrations than samples classified as normal, corroborating the hypothesis of Klasing (2008), which states that degeneration generates a calcification process in the bird's musculature. The samples affected by the severe degree of White Striping myopathy showed higher ($P = 0.0001$) sodium concentrations compared to normal samples and those affected by the moderate degree of myopathy. We can observe that the concentration of calcium ($P = 0.0013$), magnesium ($P = 0.0452$), and sodium ($P = 0.0298$) decreased with the time of storage, due to the leaching of minerals by the meat exudate produced during thawing. Chemically, the minerals associated with the reduction of free water are water-soluble substances, such as proteins, unlike lipids, which are hydrophobic and are not lost in the exudate (Cobos and Díaz, 2014), in this way, when the water comes out at the time of thawing, these minerals (calcium, magnesium, and sodium) are dragged through the water, thus reducing the concentration of minerals in the meat. There was no effect ($P = 0.9910$) of myopathy on the sulfur concentration in chicken breast meat, which it may be an indication that White Striping myopathy does not cause the process of muscle fibrosis, because when there is fibrosis in the muscle, the muscle tissue is replaced by connective tissue formed by chondroitin which is rich in sulfur.

There was an interaction ($P < 0.05$) between the severity degree of White Striping myopathy and the freezing period for the amount of phosphorus and potassium (Table 9). Samples affected by White Striping myopathy showed lower concentration of phosphorus and potassium and freezing reduced these minerals over 12 mo.

Phosphorus participates in the formation process of bone structure and the formation of cell membranes (Runho et al., 2001), with a lower concentration in meat with myopathy and reduction during freezing storage, resulting in meat with greater tenderness, a fact found in the results obtained in the shear strength analysis (Table 3).

The lower levels of potassium in meats with myopathy and frozen meats possibly caused the alteration of the muscular membranes' structure, decreasing the permeability of these, providing meat with lower values for the shear strength. Since potassium contributes to the protein synthesis process (Cavinatto, 2000) and meats with the myopathy showed lower levels, the protein synthesis was impaired, providing meats with less protein (Table 10).

In the case of storage by freezing, apparently there was a reduction in the concentration of phosphorus and potassium due to these minerals being water-soluble, the same being lost in the exudate during the process of thawing the meat.

Regarding the total coliform count (Table 11), Brazilian legislation accepts up to 104 NMP/g (BRASIL, 2001), so the studied samples had a number of total and thermotolerant coliforms within the normal range for the human consumption even after 12 mo of freezing. In another study evaluating chicken breast fillets with White Striping, total coliforms values were below the limit recommended by the Brazilian legislation (Mendes et al., 2020). The same happened for the analysis of *Staphylococcus* sp., whose permitted limit is up to 107 CFU/g, and *Salmonella* sp., whose result should indicate the absence of the bacteria in 25 g of sample (BRASIL, 2001). Regarding the total coliforms count (Table 11), Brazilian legislation accepts up to 104 NMP / g (BRASIL, 2001), therefore, it is concluded that the samples studied had a number of total and thermotolerant coliforms within the normal range for human consumption even after 12 m of freezing. The same occurred for the analysis of *Staphylococcus* sp., whose allowed limit is up to 107 CFU / g and *Salmonella* sp., whose result must indicate the absence of the bacteria in 25 g of sample (BRASIL, 2001). In another study, chicken breast fillets with pectoral myopathy, frozen for 12 mo, showed values of total coliforms and thermotolerant coliforms, Staphylococcus sp. and Salmonella sp., below the limit recommended by Brazilian legislation

Table 9. Mean values of phosphorus and potassium of breast meat from broilers affected by White Striping myopathy frozen for up to 12 mo.

	Phosphorus (g/Kg)				
	SD (n = 20)				
ST (n = 20)	Normal	Moderate	Severe		
Beginning	10.17 ± 0.17^{Aa}	9.90 ± 0.19^{Ab}	9.47 ± 0.23^{Ac}		
3 mo	9.52 ± 0.15^{Ba}	9.64 ± 0.18^{Ca}	9.31 ± 0.16^{Bb}	(SD)	<0.0001
6 mo	9.18 ± 0.21^{Cb}	9.41 ± 0.18^{Da}	8.97 ± 0.17^{Dc}	(ST)	<0.0001
9 mo	9.50 ± 0.18^{Ba}	9.49 ± 0.14^{Ca}	9.15 ± 0.19^{Cb}	(SD x ST)	<0.0001
12 mo	8.94 ± 0.14^{Dc}	9.79 ± 0.16^{Ba}	9.43 ± 0.18^{Bb}		
	Potassium (g/Kg)				
	SD (n = 20)				
ST (n = 20)	Normal	Moderate	Severe		
Beginning	12.42 ± 0.33^{Aa}	12.21 ± 0.35^{Ab}	11.51 ± 0.26^{Ac}		
3 mo	11.50 ± 0.38^{Ba}	10.92 ± 0.31^{Bb}	10.71 ± 0.42^{Bc}	(SD)	<0.0001
6 mo	11.14 ± 0.47^{Ca}	10.54 ± 0.59^{Cb}	10.31 ± 0.45^{Cc}	(ST)	<0.0001
9 mo	11.08 ± 0.40^{Da}	10.40 ± 0.37^{Db}	10.13 ± 0.41^{Dc}	(SD × ST)	<0.0001
12 mo	10.46 ± 0.30^{Ea}	9.74 ± 0.34^{Eb}	9.39 ± 0.37^{Ec}		

Means followed by different letters in the columns (upper case) and in the lines (lower case), are significantly different by the Bonferroni test ($P < 0.05$). Abbreviations: SD, severity degree; ST, storage time; SD × ST, interaction.

Table 10. Mean values of moisture, crude protein, mineral matter, and lipid oxidation (TBARS) of breast meat from broilers affected by White Striping myopathy frozen for up to 12 mo.

	Moisture (%)				
	SD (n = 20)				
ST (n = 20)	Normal	Moderate	Severe		
Beginning	71.63 ± 1.05^{Ac}	72.60 ± 0.88^{Ab}	74.57 ± 0.99^{Aa}		
3 mo	70.66 ± 1.07^{Bc}	71.75 ± 0.87^{Bb}	72.23 ± 1.25^{Ba}	(SD)	<0.0001
6 mo	69.04 ± 1.11^{Cc}	70.70 ± 1.12^{Cb}	71.68 ± 1.03^{Ca}	(ST)	<0.0001
9 mo	68.99 ± 0.96^{Dc}	69.87 ± 1.06^{Db}	70.57 ± 1.20^{Da}	(SD × ST)	<0.0001
12 mo	67.63 ± 0.99^{Ec}	68.54 ± 1.15^{Eb}	69.07 ± 1.14^{Ea}		
	Protein (%)				
	SD (n = 20)				
ST (n = 20)	Normal	Moderate	Severe		
Beginning	26.97 ± 1.01^{Aa}	24.66 ± 1.38^{Ab}	22.14 ± 1.22^{Ac}		
3 mo	24.58 ± 1.04^{Ba}	22.77 ± 1.87^{Bb}	20.62 ± 1.57^{Bc}	(SD)	<0.0001
6 mo	23.22 ± 1.36^{Ca}	21.97 ± 1.45^{Cb}	19.09 ± 1.27^{Cc}	(ST)	<0.0001
9 mo	21.26 ± 1.31^{Da}	19.45 ± 1.28^{Db}	17.70 ± 1.19^{Dc}	(SD × ST)	<0.0001
12 mo	20.12 ± 1.71^{Ea}	18.20 ± 1.29^{Eb}	16.02 ± 1.52^{Ec}		
	Mineral matter (%)				
	SD (n = 20)				
ST (n = 20)	Normal	Moderado	Severo		
Beginning	2.54 ± 0.38^{Aa}	2.27 ± 0.40^{Ab}	2.04 ± 0.32^{Ac}		
3 months	2.10 ± 0.35^{Ba}	1.99 ± 0.36^{Bb}	1.82 ± 0.31^{Bc}	(SD)	<0.0001
6 months	1.72 ± 0.45^{Ca}	1.64 ± 0.34^{Cb}	1.58 ± 0.33^{Cc}	(ST)	<0.0001
9 months	1.63 ± 0.31^{Da}	1.55 ± 0.31^{Db}	1.40 ± 0.35^{Dc}	(SD x ST)	<0.0001
12 months	1.44 ± 0.37^{Ea}	1.32 ± 0.36^{Eb}	1.19 ± 0.34^{Ec}		
	TBARS (mg MDA / kg)				
	SD (n = 20)				
ST (n = 20)	Normal	Moderado	Severo		
Beginning	0.29 ± 0.033^{Ec}	0.39 ± 0.047^{Eb}	0.43 ± 0.052^{Ea}		
3 mo	0.42 ± 0.089^{Dc}	0.45 ± 0.055^{Db}	0.61 ± 0.064^{Da}	(SD)	<0.0001
6 mo	0.61 ± 0.083^{Cc}	0.68 ± 0.055^{Cb}	0.72 ± 0.053^{Ca}	(ST)	<0.0001
9 mo	0.73 ± 0.056^{Bc}	0.82 ± 0.070^{Bb}	0.83 ± 0.060^{Ba}	(SD × ST)	<0.0001
12 mo	0.78 ± 0.053^{Ac}	0.87 ± 0.061^{Ab}	0.96 ± 0.071^{Aa}		

Means followed by different letters in the columns (upper case) and in the lines (lower case) are significantly different by the Bonferroni test ($P < 0.05$). Abbreviations: SD, severity degree; ST, storage time; SD × ST, interaction.

Table 11. Mean values of the count of total and thermotolerant coliforms, *Staphylococcus* sp. and *Salmonella* sp. of breast meat from broilers affected by White Striping myopathy frozen for up to 12 mo.

Total Coliforms (NMP/g)			
Period	Normal	Moderate	Severe
Beginning	1.1×10^1	9.2×10^0	2.3×10^1
12 mo	2.3×10^1	10×10^1	2.7×10^1

Thermotolerant (NMP/g)			
Period	Normal	Moderate	Severe
Beginning	$< 3.0 \times 10^0$	$< 3.0 \times 10^0$	$< 3.0 \times 10^0$
12 mo	$< 3.0 \times 10^0$	$< 3.0 \times 10^0$	$< 3.0 \times 10^0$

Staphylococcus sp. (UFC/g)			
Period	Normal	Moderate	Severe
Beginning	3.6×10^4	$< 1.0 \times 10^2$	$< 1.0 \times 10^2$
12 mo	5.0×10^2	1.7×10^5	3.5×10^4

Salmonella sp. (25 g)			
Period	Normal	Moderate	Severe
Beginning	Absent	Absent	Absent
12 mo	Absent	Absent	Absent

MPN/g: most likely number in 1 g of sample.
CFU/g: colony forming unit in 1 g of sample.

(Cayllahua, 2020). With these results, we can see that the meat continues to be safe for human consumption, as it contains a bacterial count within the safe limits for human consumption.

A significant part of the broiler breeding is affected by white striping myopathy, regardless of the severity degree, which may imply differences in sensory quality and impair the product appreciation. In this sense, chicken samples affected by the white striping produce less exudate, which is good for processing chicken meat. During the freezing process, a progressive increase in cooking weight loss and reduction in protein content, mineral matter, moisture and collagen content may be observed. However, these variations do not compromise the consumption of cut chicken breast meat kept frozen for up to 12 mo from a chemical and microbiological point of view. The freezing period for 12 mo at −20°C in breasts with or without myopathy increased the MFI and the sarcomere length, which provided greater meat tenderness, which suggests that the freezing process may be of great importance for the poultry industry, especially when used for breasts affected by white striping myopathy.

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DISCLOSURES

The authors have no conflicts of interest to report.

SUPPLEMENTARY MATERIALS

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