

# Physical and chemical characteristics of meat from broilers raised in 4 different rearing systems, stored under freezing for up to 12 months

A. Giampietro-Ganeco,<sup>\*,1</sup> C. M. Owens,<sup>†</sup> J. L. M. Mello,<sup>\*</sup> R. A. Souza,<sup>\*</sup> F. B. Ferrari,<sup>\*</sup> P. A. Souza,<sup>\*</sup> and H. Borba<sup>\*</sup>

<sup>\*</sup>Department of Technology, São Paulo State University – UNESP, Via de Acesso Professor Paulo Donato Castellane, s/n, Zona Rural, 14884–900, Jaboticabal-SP, Brazil; and <sup>†</sup>Department of Poultry Science, University of Arkansas, Fayetteville 72701

**ABSTRACT** This study evaluated the effects of freezing ( $-18^{\circ}\text{C}$ ) for 12 mo on attributes related to the texture of breast, drumstick, and thigh from broilers raised in 4 different rearing systems. Five-hundred carcasses of male broilers raised in 4 rearing systems (Antibiotic-free, Cobb 500,  $n = 125$ ; Free-range, Hubbard ISA,  $n = 125$ ; Conventional, Cobb 500,  $n = 125$ ; Organic, Cobb 500,  $n = 125$ ) were divided into breast, drumstick, and thigh and stored under freezing ( $-18^{\circ}\text{C}$ ) for 3, 6, 9, and 12 months. Breast, drumstick, and thigh meat from broilers raised in all studied rearing systems showed reduction ( $P < 0.001$ ) of water-holding capacity (WHC) during freezing for up to 12 months. It was observed an increase ( $P < 0.001$ ) of cooking loss in breast samples from antibiotic-free, conventional, and organic broilers,

and in thigh samples from broilers raised in all rearing systems studied. Breast meat from alternative broilers showed an increase in shear force values, while breast meat from conventional broilers became tenderer during the freezing storage. In general, alternative broilers had harder thigh meat than conventional broilers. A reduction ( $P < 0.001$ ) in myofibrillar fragmentation index and total collagen concentration was verified in breast, drumstick, and thigh samples throughout the experiment. The freezing for up to 12 mo affects characteristics related to the succulence of chicken meat. Freezing chicken meat cuts for long periods, regardless of the rearing system, may interfere with the meat texture during preparation and consumption and, consequently, influence the consumer decision in a next purchase.

**Key words:** antibiotic-free, free-range, meat cuts, organic, shelf life

2017 Poultry Science 96:3796–3804  
http://dx.doi.org/10.3382/ps/pep183

## INTRODUCTION

The deficiency of animal protein in several parts of the world has required producers and industry increasingly to adopt technologies that provide maximum production at the lowest cost, in a shorter time, and using the smallest possible area. As a result, poultry farming has become one of the most efficient activities in animal protein production, with excellent performance in transforming plant products into foods with high protein value for human consumption. Brazil currently ranks second in the world chicken meat production ranking and is the largest exporter, having produced 13.14 million tons in 2015 and exporting 4.3 million tons of meat (ABPA, 2016).

The marketing of chicken meat has changed considerably in respect to the way that the product is presented. In the early 90s, most of the poultry meat exports were made in the form of whole chickens, but

nowadays, with changing consumer profile and the demand for quick and practical products, industries have focused on commercialization of selected cuts, which corresponded in 2015 to 58% of the total chicken meat exported by Brazil (ABPA, 2016).

With the growing consumer concern about acquiring healthful and naturally grown food, alternative chicken rearing is on the rise in Brazil (Hardy et al., 2013) because it is considered environmentally correct, associated with bird health and animal welfare standards (Castellini et al., 2002a), which makes it an important factor to define food preference and purchase decision (Fanatico et al., 2009; Hardy et al., 2013). The belief that alternative poultry offer meat of better quality, taste, and nutritional composition makes consumers prefer this kind of meat over meat from conventional broilers (Hardy et al., 2013).

With the new tendencies of the meat market, the processing industries started to offer differentiated products that can add value to the poultry meat (Fanatico et al., 2007). Freezing has become an essential tool for preserving meat by providing transport and storage for long periods (Carroll and Alvarado, 2008; Demirok et al., 2013), preserving the food quality. So, this study

© 2017 Poultry Science Association Inc.

Received July 26, 2016.

Accepted June 13, 2017.

<sup>1</sup>Corresponding author: [algamp@yahoo.com.br](mailto:algamp@yahoo.com.br)

**Table 1.** Composition and calculated nutrient content of diets (as-fed basis).

Ingredient (%)	Conventional broilers			Antibiotic-free, free-range, and organic broilers		
	Starter	Growing	Finisher	Starter	Growing	Finisher
Corn grain	57.2	63.9	65.0	60.2	67.8	71.5
Soybean meal	36.9	30.3	27.9	36.1	27.7	23.5
Soybean oil	1.8	2.5	3.9	0	1.00	2.00
Dicalcium phosphate	1.8	1.6	1.4	1.9	1.7	1.5
Calcitic limestone	1.3	0.8	0.9	1.1	1.2	1.4
Salt	0.3	0.3	0.3	0.4	0.3	0.3
Vitamin and mineral mix*	0.50	0.50	0.50	0.20	0.20	0.20
DL- methionine (98%)	0.12	0.10	0.10	0.08	0.05	0.05
Calculated nutrient composition						
Crude protein (%)	21.5	19.0	18.0	23.7	23.5	17.0
ME (MJ/kg)	12.6	13.1	13.5	12.3	12.8	13.3
Available phosphorus (%)	0.45	0.40	0.30	0.70	0.60	0.50
Calcium (%)	0.95	0.84	0.80	1.00	1.00	0.90
Total M+C (%)	0.85	0.78	0.75	0.83	0.78	0.73
Total methionine (%)	0.50	0.46	0.45	0.51	0.48	0.42
Total Lysine (%)	1.2	1.1	1.0	1.2	1.1	1.0

ME: Metabolizable energy; M+C: methionine + cysteine; Dry matter of the as-fed diets: 90%; \*Product composition (per kg) – A vitamin - 150.000 UI, D3 vitamin - 35.000 UI, E vitamin - 480 mg, K vitamin - 110 mg, B2 vitamin - 170 mg, B6 vitamin - 70 mg, B12 vitamin - 650 mcg, B3 vitamin - 700 mg, biotin - 3 mg, pantothenic acid - 500 mg, folic acid - 25 mg, C vitamin - 12 mg, Fe - 1.100 mg, Cu - 300 mg, I - 24 mg, methionine - 20 mg, Ca - 176 mg, P - 60 mg, Na - 23 mg, Cl - 36 mg, <sup>x</sup>growth promoter - 2 mg, <sup>y</sup>coccidiostatic - 10g, <sup>z</sup>BHT - 1 mg, Mg - 5 g, S - 4 g, inert vehicle - 1.000 g. Dry matter of the as-fed diets: 90%.

<sup>x,y,z</sup>Not added to the diet provided to antibiotic-free, free-range and organic broilers.

aimed to evaluate the effects of freezing ( $-18^{\circ}\text{C}$ ) for 12 mo (validity period determined by the industry for frozen chicken meat cuts) on attributes related to the texture of breast, drumstick, and thigh from broilers raised in 4 different rearing systems.

## MATERIALS AND METHODS

This study was performed at the Laboratory of Animal Products Technology of the São Paulo State University/UNESP, located in Jaboticabal, São Paulo, Brazil ( $21^{\circ}08' \text{ S}$ ,  $48^{\circ}11' \text{ W}$ , 583 m altitude).

### Samples and Experimental Procedures

Five-hundred carcasses of male broilers raised in 4 rearing systems (Antibiotic-free, Cobb 500,  $n = 125$ ; Free-range, Hubbard ISA,  $n = 125$ ; Conventional, Cobb 500,  $n = 125$ ; Organic, Cobb 500,  $n = 125$ ) were used in this study. Samples were taken from commercial slaughterhouses located in the state of São Paulo, Brazil, inspected by the Brazilian Federal Inspection Service (SIF).

In the antibiotic-free production system, broilers were reared in conventional sheds, with no access to grazing area, at a maximum density of  $30 \text{ kg/m}^2$ , but the use of antibiotics (either as therapeutic agents or as performance enhancers) or chemotherapeutic or anticoccidial agents and animal ingredients in food is not allowed. Broilers raised in the antibiotic-free system were slaughtered at 45 d of age. In the free-range

system, broilers were reared in conventional sheds, with a maximum density of  $30 \text{ kg/m}^2$ , and from 28 d of age, birds had access to grazing with a minimum density of  $3 \text{ m}^2$  of available area for each bird. According to Brazilian regulations, the use of performance enhancers, chemotherapeutic agents, or animal ingredients in food is not allowed in the free-range farming system. Free-range broilers were slaughtered at 85 d of age. In the conventional rearing system, broilers were confined in conventional sheds for chicken rearing where they were fed a diet containing performance enhancers, at a maximum density of  $40 \text{ kg/m}^2$ . Conventional broilers were slaughtered at 42 d of age. In the organic system, broilers were raised in a semi-confined environment with free access to grazing from 25 d of age, at a maximum density of  $30 \text{ kg/m}^2$ . Broilers were fed a diet containing certified organic ingredients, devoid of animal ingredients and antibiotics, and were slaughtered at 48 d of age. Broilers were fed the diets shown in Table 1.

In the slaughterhouse, all broiler carcasses from all rearing systems were collected following the routine procedures of commercial slaughtering, meeting the requirements of animal welfare and regulations of the FIS of the Ministry of Agriculture, Livestock and Supply (MAPA). After stabilization of rigor mortis (4 h after slaughter), the 500 carcasses were divided into the main commercial cuts (breast, thigh, and drumstick). The slaughter ages and average weights of the breast, thigh, and drumstick for the 4 different rearing systems are shown in Table 2.

On the d of slaughter, 100 carcasses ( $n = 25$  carcasses for each rearing system) were analyzed

**Table 2.** Slaughter age and average weight of breast, thigh, and drumstick for the 4 different rearing systems.

System	Slaughter age	Average weight (g)		
		Breast <sup>x</sup>	Drumstick <sup>y</sup>	Thigh <sup>y</sup>
Antibiotic-free	45 d of age	796.7	285.3	384.5
Free-range	85 d of age	678.3	369.3	479.3
Conventional	42 d of age	1181.6	304.6	343.8
Organic	48 d of age	696.5	299.3	397.9

<sup>x</sup>Average weight of the breast with bone.<sup>y</sup>Sum of the average weights of the right and left cuts.

considering each sample, a broiler carcass divided into breast, drumstick, and thigh, which were only chilled (4°C) and not subjected to any freezing process. The other samples (400 carcasses divided into breast, thigh, and drumstick;  $n = 1\ 200$  cuts) were frozen in a freezing tunnel (-35°C) for 6 h, individually packaged in plastic bags (18 microns; without vacuum), and then samples were stored under freezing (-18°C) (RVC-1400-4P, Cozil, Itaquaquecetuba-SP, Brazil) until completing the proposed freezing time for analysis (3 mo,  $n = 25$  for each cut, in each rearing system,  $n = 300$ ; 6 mo,  $n = 25$  for each cut, in each rearing system,  $n = 300$ ; 9 mo,  $n = 25$  for each cut, in each rearing system,  $n = 300$ ; and 12 mo  $n = 25$  for each cut, in each rearing system,  $n = 300$ ).

At the end of each freezing period, samples were thawed overnight under refrigeration (4°C) (Cozil, RVC-1400-4P, Itaquaquecetuba-SP, Brazil) for further analysis. The *pectoralis major*, *fibularis longus* and *biceps femoris* muscles (Baumel et al., 1993) were used for the analyses of breast, drumstick, and thigh, respectively. Samples of the right and left sides were used for the analyses of drumstick and thigh meat.

## Methods

The following meat quality parameters were performed in breast, drumstick, and thigh meat from broilers raised in the 4 rearing systems studied: water-holding capacity, cooking loss, shear force, myofibrillar fragmentation index, and total collagen.

## Physical Analyses

Water-holding capacity was determined as described by Hamm (1961). In summary, 2 g of deboned muscle were placed between 2 filter papers and acrylic plates and subjected to the pressure exerted by a 10 kg weight, for 5 minutes. Subsequently, samples were weighed again to determine water-holding capacity, expressed as a percentage, according to the formula:  $(\text{Final Weight} \times 100)/\text{Initial Weight}$ .

Cooking loss was determined in deboned and skinned samples (breast, drumstick, and thigh) according to Honikel (1987). Samples with 6 cm width and 80 g, on average, were weighed, packaged in plastic bags (without vacuum), and cooked in a water bath at 85°C for

30 minutes. After cooling at room temperature, samples were re-weighed to determine the cooking loss according to the formula:  $[(\text{Initial Weight} - \text{Final Weight})/\text{Initial Weight}] \times 100$ . Three sub-samples with a cross-sectional area equal to 1 cm<sup>2</sup> and approximately 3 cm in length were obtained from each cooked sample to determine the shear force. The sub-samples were placed with their fibers oriented perpendicularly to a Warner-Bratzler device (HDP/BSW Warner Bratzler with crosshead speed 5 mm/s) coupled to a texture analyzer (TA-XT2i, Stable Micro Systems, Ltd, Godalming, UK), and were subjected to cutting (Lyon et al., 1998). The force required for shearing was expressed in Newton (N).

## Chemical Analyses

The myofibrillar fragmentation index was determined as described by Culler et al. (1978). From each frozen sample, we obtained 3 g subsamples that were chopped with a scalpel to remove any visible fat and connective tissue. Subsequently, samples were homogenized in a Turrax stirring device (MA 102, Marconi Laboratory Equipment LTD., Piracicaba-SP, Brazil) for 1.5 min, with 30 mL of extraction buffer. Then, the homogenate was centrifuged at 14,400 g for 15 min and 4°C. After discarding the supernatant, the precipitate was dispersed in 30 mL of extraction buffer, stirred with a glass rod, and centrifuged again; this operation was repeated once more. After discarding the supernatant, 15 mL of extraction buffer were added to the precipitate, and the resulting suspension was filtered through a polyethylene strainer to remove connective tissue. The protein concentration of the myofibril suspension was determined by the biuret method as described by Gornall et al. (1949). An aliquot from the suspension of myofibrils was diluted with extraction buffer to a protein concentration of  $0.5 \pm 0.05$  mg/mL. The diluted suspension of myofibrils was stirred, and shortly thereafter, its optical density was measured in a spectrophotometer at 540 nm. The MFI was calculated according to the formula:  $\text{MFI} = \text{optical density} \times 200$ .

Total collagen was quantified via determination of hydroxyproline amino acid content according to method 990.26 from AOAC (2005). A raw meat sample weighing 4 g was digested in 3M H<sub>2</sub>SO<sub>4</sub> at 110°C for 17 hours. Fat was eliminated from the sample by adding 5 mL petroleum ether. The hydrolysate was

**Table 3.** Mean values of water-holding capacity, cooking loss, shear force, myofibrillar fragmentation index, and total collagen of breast meat from broilers raised in 4 different rearing systems, stored for 12 months.

Water-holding capacity (%)							
System (S)	Months of freezing (F)					<i>P</i> -value	
	Chilled	3 mo	6 mo	9 mo	12 mo		
Antibiotic-free	72.29 <sup>A,a</sup>	64.20 <sup>A,B,b</sup>	64.66 <sup>A,b</sup>	66.12 <sup>A,b</sup>	61.19 <sup>A,c</sup>	<i>P</i> (F)	<0.001
Free-range	65.54 <sup>B,a</sup>	66.37 <sup>A,a</sup>	64.73 <sup>A,a</sup>	60.44 <sup>B,b</sup>	62.45 <sup>A,a,b</sup>	<i>P</i> (S)	<0.001
Conventional	72.21 <sup>A,a</sup>	61.92 <sup>B,b</sup>	59.06 <sup>B,b</sup>	55.96 <sup>C,c</sup>	59.61 <sup>A,B,b</sup>	<i>P</i> (F×S)	<0.001
Organic	65.16 <sup>B,a</sup>	68.25 <sup>A,a</sup>	66.93 <sup>A,a</sup>	62.00 <sup>B,a</sup>	57.94 <sup>B,b</sup>		
Cooking loss (%)							
Antibiotic-free	21.93 <sup>B,c</sup>	25.43 <sup>B,b</sup>	29.09 <sup>A,B,a</sup>	29.80 <sup>A,a</sup>	29.68 <sup>B,a</sup>	<i>P</i> (F)	<0.001
Free-range	26.92 <sup>A,a</sup>	27.26 <sup>B,a</sup>	28.18 <sup>B,a</sup>	27.35 <sup>B,a</sup>	28.39 <sup>B,a</sup>	<i>P</i> (S)	<0.001
Conventional	27.51 <sup>A,b</sup>	30.21 <sup>A,a</sup>	30.04 <sup>A,B,a</sup>	30.41 <sup>A,a</sup>	31.30 <sup>B,a</sup>	<i>P</i> (F×S)	<0.001
Organic	28.74 <sup>A,b</sup>	31.05 <sup>A,a</sup>	32.15 <sup>A,a</sup>	30.26 <sup>A,a,b</sup>	33.74 <sup>A,a</sup>		
Shear force (N)							
Antibiotic-free	19.32 <sup>A,b</sup>	15.40 <sup>A,B,b</sup>	18.04 <sup>A,B,b</sup>	24.42 <sup>A,a</sup>	24.03 <sup>A,a</sup>	<i>P</i> (F)	<0.001
Free-range	17.75 <sup>A,b</sup>	19.22 <sup>A,b</sup>	20.59 <sup>A,a,b</sup>	23.34 <sup>A,a</sup>	22.65 <sup>A,a</sup>	<i>P</i> (S)	<0.001
Conventional	20.20 <sup>A,a</sup>	12.65 <sup>B,b</sup>	12.65 <sup>B,b</sup>	12.65 <sup>B,b</sup>	13.83 <sup>B,b</sup>	<i>P</i> (F×S)	<0.001
Organic	18.24 <sup>A,a,b</sup>	14.02 <sup>B,b</sup>	15.79 <sup>B,b</sup>	16.38 <sup>A,B,b</sup>	21.48 <sup>A,a</sup>		
Myofibrillar fragmentation index							
Antibiotic-free	126.58 <sup>A,B,a</sup>	139.38 <sup>C,a</sup>	136.24 <sup>B,a</sup>	114.58 <sup>B,a</sup>	91.48 <sup>B,b</sup>	<i>P</i> (F)	<0.001
Free-range	118.13 <sup>B,c</sup>	161.85 <sup>A,a</sup>	153.26 <sup>A,b</sup>	151.81 <sup>A,b</sup>	108.02 <sup>B,d</sup>	<i>P</i> (S)	<0.001
Conventional	141.48 <sup>A,b</sup>	169.53 <sup>A,a</sup>	119.24 <sup>B,c</sup>	122.66 <sup>B,c</sup>	91.39 <sup>B,d</sup>	<i>P</i> (F×S)	<0.001
Organic	117.25 <sup>B,d</sup>	148.65 <sup>B,b</sup>	159.01 <sup>A,a</sup>	140.51 <sup>A,c</sup>	149.75 <sup>A,b</sup>		
Total collagen (%)							
Antibiotic-free	3.52 <sup>B,a</sup>	3.57 <sup>B,a</sup>	3.53 <sup>A,a</sup>	2.65 <sup>A,b</sup>	2.09 <sup>A,b</sup>	<i>P</i> (F)	<0.001
Free-range	5.53 <sup>A,a</sup>	2.97 <sup>B,b</sup>	2.66 <sup>B,b,c</sup>	2.22 <sup>B,b,c</sup>	2.03 <sup>A,c</sup>	<i>P</i> (S)	<0.001
Conventional	3.75 <sup>B,a</sup>	3.42 <sup>B,a</sup>	3.18 <sup>A,a,b</sup>	2.18 <sup>B,b</sup>	2.77 <sup>A,b</sup>	<i>P</i> (F×S)	<0.001
Organic	5.53 <sup>A,a</sup>	4.42 <sup>A,b</sup>	3.89 <sup>A,b</sup>	2.80 <sup>A,c</sup>	2.12 <sup>A,c</sup>		

<sup>A-C</sup>Means followed by different uppercase letters in each column are significantly different according to Tukey's test ( $P < 0.05$ ).

<sup>a-d</sup>Means followed by different lowercase letters in each row are significantly different according to Tukey's test ( $P < 0.05$ ).

System: Rearing system ( $n = 125/\text{system}$ ). Months of freezing ( $n = 100/\text{time of frozen storage}$ ).

filtered, and then 5 mL were transferred to 100 mL volumetric flasks. From the new dilution, 4 mL aliquots were transferred to test tubes and mixed with 2 mL oxidation reagent (Chloramine-T 1.41%) and 2 mL color reagent (prepared by dissolving 10 g of p-dimethylaminobenzaldehyde in 35 mL 60% perchloric acid and 60 mL of isopropanol). After stirring, the tubes were heated in a water bath (60°C) for 15 minutes. The readings were performed on a spectrophotometer at 558 nm. A standard curve was constructed using a solution with known concentrations of L-hydroxyproline. The total collagen concentration was estimated as 8 times the hydroxyproline concentration.

## Statistical Analysis

Data on water-holding capacity, cooking loss, shear force, myofibrillar fragmentation index, and total collagen were evaluated in a  $4 \times 5$  completely randomized factorial design (4 rearing systems and 5 freezing periods) with 25 repetitions using the General Linear Models procedure of the Statistical Analysis System (SAS Institute Inc, 2002–2003). Breast, drumstick, and thigh

muscles were statistically analyzed separately. All data were tested by analysis of variance and compared by Tukey's test with a significance level of  $P < 0.05$ .

## RESULTS

### Breast Meat

Breast meat from broilers raised in all studied systems showed reduction ( $P < 0.001$ ) in water-holding capacity during storage under freezing, with the highlight of breast meat from organic broilers, which had water-holding capacity reduced just after 12 mo of storage (Table 3). With the reduction of the water-holding capacity, the muscle loses the ability to hold water within the cells and thereby can lead to loss of nutritional value due to the exudate from the muscles. Thus, when moisture is lost, consequently the tenderness and flavor are affected, resulting in drier and tougher meat. Breast meat from broilers raised in the conventional rearing system showed lower water-holding capacity when freezer-stored for 3, 6, and 9 months.

Regarding of cooking loss, an increase ( $P < 0.001$ ) of cooking loss values during storage under freezing in



**Table 4.** Mean values of water-holding capacity, cooking loss, shear force, myofibrillar fragmentation index, and total collagen of drumstick meat from broilers raised in 4 different rearing systems, stored for 12 months.

Water-holding capacity (%)							
System (S)	Months of freezing (F)					<i>P</i> -value	
	Chilled	3 mo	6 mo	9 mo	12 mo		
Antibiotic-free	71.48 <sup>B,a</sup>	70.18 <sup>B,a</sup>	68.08 <sup>B,a,b</sup>	67.18 <sup>B,b</sup>	68.84 <sup>A,a,b</sup>	<i>P</i> (F)	<0.001
Free-range	75.83 <sup>A,a</sup>	72.58 <sup>B,b</sup>	73.98 <sup>A,a,b</sup>	70.76 <sup>A,c</sup>	71.08 <sup>A,c</sup>	<i>P</i> (S)	<0.001
Conventional	74.22 <sup>A,a</sup>	70.26 <sup>A,a</sup>	66.98 <sup>B,b</sup>	66.85 <sup>B,b</sup>	63.25 <sup>B,c</sup>	<i>P</i> (F×S)	<0.001
Organic	72.78 <sup>B,a</sup>	64.37 <sup>C,b</sup>	64.47 <sup>C,b</sup>	65.28 <sup>B,b</sup>	66.32 <sup>A,b</sup>		
Cooking loss (%)							
Antibiotic-free	32.89 <sup>B,a</sup>	35.38 <sup>A,a</sup>	34.20 <sup>A,a</sup>	33.70 <sup>A,a</sup>	33.49 <sup>B,a</sup>	<i>P</i> (F)	<0.001
Free-range	30.33 <sup>C,b</sup>	32.96 <sup>B,a</sup>	31.18 <sup>B,a,b</sup>	30.36 <sup>B,b</sup>	31.49 <sup>B,a</sup>	<i>P</i> (S)	<0.001
Conventional	27.12 <sup>D,b</sup>	33.51 <sup>A,B,a</sup>	33.67 <sup>A,a</sup>	29.86 <sup>B,a</sup>	31.89 <sup>B,a</sup>	<i>P</i> (F×S)	<0.001
Organic	34.51 <sup>A,a,b</sup>	36.36 <sup>A,a</sup>	35.98 <sup>A,a</sup>	32.59 <sup>B,b</sup>	37.01 <sup>A,a</sup>		
Shear force (N)							
Antibiotic-free	28.34 <sup>A,a</sup>	28.83 <sup>A,a</sup>	14.12 <sup>A,b</sup>	15.30 <sup>A,b</sup>	13.92 <sup>C,b</sup>	<i>P</i> (F)	<0.001
Free-range	24.03 <sup>A,B,a</sup>	21.77 <sup>A,a</sup>	27.95 <sup>A,a</sup>	14.81 <sup>A,b</sup>	18.44 <sup>B,a,b</sup>	<i>P</i> (S)	<0.001
Conventional	18.44 <sup>B,a</sup>	19.23 <sup>B,a</sup>	19.15 <sup>C,a</sup>	13.34 <sup>A,b</sup>	14.41 <sup>C,b</sup>	<i>P</i> (F×S)	<0.001
Organic	28.05 <sup>A,a</sup>	21.77 <sup>A,B,b</sup>	18.04 <sup>B,b</sup>	16.57 <sup>A,b</sup>	21.48 <sup>A,b</sup>		
Myofibrillar fragmentation index							
Antibiotic-free	115.39 <sup>B,a</sup>	121.83 <sup>A,a</sup>	109.59 <sup>A,a</sup>	92.45 <sup>B,b</sup>	91.43 <sup>A,B,b</sup>	<i>P</i> (F)	<0.001
Free-range	131.24 <sup>A,B,a</sup>	125.26 <sup>A,b</sup>	123.80 <sup>A,b</sup>	92.45 <sup>B,c</sup>	95.03 <sup>A,B,c</sup>	<i>P</i> (S)	<0.001
Conventional	140.66 <sup>A,a</sup>	104.27 <sup>B,b</sup>	88.82 <sup>B,c</sup>	88.46 <sup>B,c</sup>	85.75 <sup>B,c</sup>	<i>P</i> (F×S)	<0.001
Organic	143.61 <sup>A,a</sup>	118.77 <sup>A,b</sup>	119.57 <sup>A,b</sup>	113.46 <sup>A,b</sup>	94.49 <sup>A,c</sup>		
Total collagen (%)							
Antibiotic-free	5.20 <sup>B,a</sup>	2.48 <sup>B,b</sup>	2.86 <sup>B,b</sup>	2.81 <sup>B,b</sup>	3.08 <sup>B,b</sup>	<i>P</i> (F)	<0.001
Free-range	3.18 <sup>C,a</sup>	2.16 <sup>B,C,b</sup>	1.73 <sup>C,b</sup>	1.44 <sup>C,b</sup>	1.77 <sup>C,b</sup>	<i>P</i> (S)	<0.001
Conventional	6.73 <sup>A,a</sup>	6.58 <sup>A,b</sup>	5.98 <sup>A,b</sup>	5.96 <sup>A,b</sup>	6.00 <sup>A,b</sup>	<i>P</i> (F×S)	<0.001
Organic	4.62 <sup>B,a</sup>	2.55 <sup>B,b</sup>	1.43 <sup>C,c</sup>	1.70 <sup>C,b,c</sup>	1.15 <sup>C,c</sup>		

<sup>A-D</sup> Means followed by different uppercase letters in each column are significantly different according to Tukey's test ( $P < 0.05$ ).

<sup>a-c</sup> Means followed by different lowercase letters in each row are significantly different according to Tukey's test ( $P < 0.05$ ).

System: Rearing system ( $n = 125/\text{system}$ ). Months of freezing ( $n = 100/\text{time of frozen storage}$ ).

breast samples from antibiotic-free, conventional, and organic broilers was observed. Before storage, there was no significant difference ( $P > 0.05$ ) among chilled samples for shear force, and from the third month of freezing, it was observed that breast meat from conventional and organic broilers showed lower ( $P < 0.001$ ) shear force values than the others, which represents tenderer meat.

Meat with more firm texture was observed from the ninth mo of freezing in samples from broilers raised in the antibiotic-free rearing system and from the sixth mo of freezing in samples from broilers raised in the free-range rearing system. Breast meat from organic broilers showed lower tenderness just at 12 mo of freezing. This may be related to ice crystals produced due to the protein denaturation, which occurs in frozen meat products, because proteins lose their ability to hold intracellular water, which interferes with the texture after thawing. Moreover, it was observed that breast meat from conventional broilers became tenderer during the freezing process analyzed through the experiment.

Regarding the myofibrillar fragmentation index, greater variation ( $P < 0.001$ ) among samples from the different studied rearing systems was observed throughout the experiment, but is important to highlight that

the myofibrillar fragmentation index was reduced in samples from broilers raised in all rearing systems studied, in the second half of the experiment. During 12 mo of freezing, the reduction ( $P < 0.001$ ) of total collagen in breast meat from conventional and alternative broilers studied was observed. Before freezing, breast samples from free-range and organic broilers, which were raised with access to grazing, showed higher ( $P < 0.001$ ) total collagen percent than breast meat from antibiotic-free and conventional broilers, which were raised confined.

### Drumstick Meat

There were variations ( $P < 0.001$ ) in water-holding capacity of drumstick meat among samples from broilers raised in the different rearing systems studied during freezing (Table 4). A reduction ( $P < 0.001$ ) in water-holding capacity during freezing was observed, from the third mo of freezing and throughout the experiment, in samples from broilers raised in all rearing systems studied.

An effect of freezing ( $P > 0.05$ ) on the cooking loss in drumstick samples from antibiotic-free broilers was not verified. Before freezing, chilled drumstick samples from

**Table 5.** Mean values of water-holding capacity, cooking loss, shear force, myofibrillar fragmentation index, and total collagen of thigh meat from broilers raised in 4 different rearing systems, stored for 12 months.

Water-holding capacity (%)							
System (S)	Months of freezing (F)					<i>P</i> -value	
	Chilled	3 mo	6 mo	9 mo	12 mo		
Antibiotic-free	72.19 <sup>A,B,a</sup>	72.28 <sup>A,a</sup>	71.19 <sup>A,a,b</sup>	69.06 <sup>A,b</sup>	71.25 <sup>A,a,b</sup>	<i>P</i> (F)	<0.001
Free-range	74.57 <sup>A,a</sup>	74.61 <sup>A,a</sup>	69.76 <sup>A,b</sup>	67.87 <sup>A,b</sup>	70.03 <sup>A,b</sup>	<i>P</i> (S)	<0.001
Conventional	71.24 <sup>A,B,b</sup>	73.20 <sup>A,a</sup>	63.78 <sup>B,c</sup>	63.40 <sup>B,c</sup>	65.54 <sup>B,c</sup>	<i>P</i> (F×S)	<0.001
Organic	69.55 <sup>B,b</sup>	74.26 <sup>A,a</sup>	70.39 <sup>A,b</sup>	68.00 <sup>A,b</sup>	70.08 <sup>A,b</sup>		
Cooking loss (%)							
Antibiotic-free	33.52 <sup>A,b</sup>	33.96 <sup>B,b</sup>	35.12 <sup>A,b</sup>	36.69 <sup>A,b</sup>	40.42 <sup>A,a</sup>	<i>P</i> (F)	<0.001
Free-range	32.17 <sup>A,a</sup>	30.65 <sup>C,b</sup>	29.00 <sup>B,b</sup>	31.30 <sup>B,a</sup>	31.51 <sup>C,a</sup>	<i>P</i> (S)	<0.001
Conventional	28.33 <sup>B,b</sup>	33.81 <sup>B,a</sup>	33.59 <sup>A,a</sup>	31.85 <sup>B,a</sup>	32.06 <sup>C,a</sup>	<i>P</i> (F×S)	<0.001
Organic	35.09 <sup>A,a</sup>	36.29 <sup>A,a</sup>	33.47 <sup>A,a,b</sup>	31.09 <sup>B,b</sup>	36.68 <sup>B,a</sup>		
Shear force (N)							
Antibiotic-free	27.56 <sup>A,a</sup>	15.49 <sup>A,b</sup>	18.93 <sup>A,b</sup>	15.20 <sup>B,b</sup>	19.32 <sup>A,b</sup>	<i>P</i> (F)	<0.001
Free-range	22.55 <sup>A,a</sup>	20.69 <sup>A,a,b</sup>	17.16 <sup>B,b</sup>	17.36 <sup>B,b</sup>	16.57 <sup>A,b</sup>	<i>P</i> (S)	<0.001
Conventional	12.06 <sup>B,a</sup>	10.10 <sup>B,a</sup>	10.88 <sup>C,a</sup>	10.10 <sup>C,a</sup>	10.69 <sup>B,a</sup>	<i>P</i> (F×S)	<0.001
Organic	29.62 <sup>A,a</sup>	14.41 <sup>A,b</sup>	17.26 <sup>B,b</sup>	22.65 <sup>A,b</sup>	21.28 <sup>A,b</sup>		
Myofibrillar fragmentation index							
Antibiotic-free	121.22 <sup>B,a</sup>	106.19 <sup>C,a</sup>	116.99 <sup>A,a</sup>	89.17 <sup>B,b</sup>	85.63 <sup>B,b</sup>	<i>P</i> (F)	<0.001
Free-range	117.99 <sup>B,a</sup>	115.84 <sup>B,a</sup>	92.80 <sup>B,b</sup>	97.95 <sup>B,b</sup>	84.62 <sup>B,c</sup>	<i>P</i> (S)	<0.001
Conventional	139.51 <sup>B,a</sup>	116.92 <sup>B,b</sup>	121.02 <sup>A,b</sup>	107.01 <sup>A,b</sup>	91.29 <sup>B,c</sup>	<i>P</i> (F×S)	<0.001
Organic	154.69 <sup>A,a</sup>	150.93 <sup>A,a</sup>	125.60 <sup>A,b</sup>	113.21 <sup>A,b</sup>	105.97 <sup>A,c</sup>		
Total collagen (%)							
Antibiotic-free	3.05 <sup>B,a</sup>	2.23 <sup>B,b</sup>	2.06 <sup>B,a</sup>	2.67 <sup>B,b</sup>	2.11 <sup>B,b</sup>	<i>P</i> (F)	<0.001
Free-range	5.65 <sup>A,a</sup>	4.62 <sup>A,b</sup>	3.76 <sup>A,b,c</sup>	3.09 <sup>B,c,d</sup>	2.25 <sup>B,d</sup>	<i>P</i> (S)	<0.001
Conventional	5.88 <sup>A,a</sup>	4.78 <sup>A,b</sup>	4.21 <sup>A,b</sup>	4.44 <sup>A,b</sup>	3.91 <sup>A,c</sup>	<i>P</i> (F×S)	<0.001
Organic	3.18 <sup>B,a</sup>	2.47 <sup>B,a,b</sup>	1.68 <sup>C,b,c</sup>	1.79 <sup>C,b,c</sup>	1.24 <sup>C,c</sup>		

<sup>A-C</sup>Means followed by different uppercase letters in each column are significantly different according to Tukey's test ( $P < 0.05$ ).

<sup>a-d</sup>Means followed by different lowercase letters in each row are significantly different according to Tukey's test ( $P < 0.05$ ).

System: Rearing system (n = 125/system). Months of freezing (n = 100/time of frozen storage).

conventional broilers showed the lowest cooking loss among samples from all studied rearing systems, probably due to their high water-holding capacity (74.22%). Considering the mo of freezing, there was variation ( $P < 0.001$ ) in cooking loss values throughout the 12 months. During the experiment, the reduction ( $P < 0.001$ ) of shear force in drumstick samples from broilers raised in all rearing systems studied was observed. There were variations ( $P < 0.001$ ) among samples from the studied rearing systems in each freezing time analyzed, over the 12-month freeze.

During 12 mo of freezing, the reduction ( $P < 0.001$ ) of the myofibrillar fragmentation index in samples from broilers raised in all rearing systems studied was observed. Variation ( $P < 0.001$ ) was observed among samples from the different studied rearing systems, considering each freezing time. Regarding total collagen percentage, the reduction ( $P < 0.001$ ) of total collagen in drumstick meat was observed from conventional and alternative broilers during 12 mo of freezing. Although they were raised in closed sheds, conventional broilers showed higher ( $P < 0.001$ ) total collagen percentage in drumstick meat throughout the experiment.

## Thigh Meat

Thigh meat from broilers raised in all studied rearing systems showed a reduction ( $P < 0.001$ ) in water-holding capacity during the freezing storage for up to 12 mo (Table 5). From the sixth mo of freezing, thigh meat from conventional broilers showed the lowest water-holding capacity among samples from all rearing systems studied. There were no statistical differences among samples from antibiotic-free, free-range, and organic rearing systems, at 6, 9, and 12 mo of freezing, regarding water-holding capacity.

Due to the reduction of water-holding capacity, it was observed that, during the storage under freezing for up to 12 mo, the cooking loss increased ( $P < 0.001$ ) in samples from broilers raised in all rearing systems studied. There was a reduction ( $P < 0.001$ ) of shear force value, which indicates an increase in tenderness, in thigh meat from broilers raised in antibiotic-free, free-range, and organic rearing systems, during storage for up to 12 months. The freezing time did not influence the tenderness of thigh meat from conventional broilers. In general, alternative broilers had harder thigh meat than conventional broilers.

As was observed in breast and drumstick meat, the MFI value of thigh meat from broilers raised in all rearing systems studied decreased ( $P < 0.001$ ) during the freezing time. In addition, there was also a reduction ( $P < 0.001$ ) in total collagen percentage in thigh meat from broilers raised in the 4 rearing systems evaluated. During the evaluation period, mainly from the ninth mo of freezing, it was observed that the thigh meat from conventional broilers had higher ( $P < 0.001$ ) total collagen percentage than the other samples studied.

## DISCUSSION

Breast, drumstick, and thigh meat from broilers raised in all studied rearing systems showed reduction ( $P < 0.001$ ) of water-holding capacity during freezing for up to 12 months. In addition, an increase ( $P < 0.001$ ) of cooking loss in breast samples was observed from antibiotic-free, conventional, and organic broilers, and in thigh samples from broilers raised in all rearing systems studied. Water-holding capacity is among the most important functional properties of meat (Mello et al., 2017a) and is defined as the capacity of meat to keep bound water during the application of forces such as heating, grinding, cutting, or compression during transport (Zhang et al., 2005). Cooking loss is directly related to the ability of meat to hold water within the cells, which can be lost in large quantities during cooking if the ability to retain it is not suitable. As the water-holding capacity decreases, losses during storage, sale, and processing increase (Lawson, 2004; Gomide et al., 2013; Oliveira et al., 2015). In the industry, storage losses are an important economic factor (Campañone et al., 2001), making higher water-holding capacity in meat samples a desirable feature, as it may directly influence the final yield.

As a consequence, with the reduction of water-holding capacity, the muscle loses the ability to hold water within the cells and thereby can lead to loss of nutritional value due to the exudate produced from muscles (Dabes, 2001). The reduction of water-holding capacity also may influence the meat color through the increase of lightness, due to the high amount of water on the meat surface (Purchas, 1990). Moreover, when moisture is lost, consequently tenderness and flavor are affected, resulting in drier and tougher meat. So, minimizing water losses is important to keep the flavor, texture, and juiciness, which are important sensory characteristics for the meat consumer (Ramos and Gomide, 2007).

Previous researches concluded that meat from broilers raised in extensive systems can show considerably reduced water-holding capacity when compared to meat from confined broilers (Castellini et al., 2002b; Fanatico et al., 2006), which can be verified in chilled breast meat from free-range and organic broilers (Table 3), and in chilled drumstick and thigh meat from organic broilers (Tables 4 and 5, respectively). Therefore, the reduction of water-holding capacity observed mainly in meat

from conventional and organic broilers, during freezing for long times, can affect consumption.

Breast meat from alternative broilers showed an increase in shear force values, while breast meat from conventional broilers became tenderer during the freezing storage. There was a reduction ( $P < 0.001$ ) of shear force in drumstick samples from broilers raised in all rearing systems studied. In general, alternative broilers had harder thigh meat than conventional broilers. During freezing, proteins lose their ability to hold intracellular water, and less water-holding capacity may result in the production of ice crystals, which may interfere with the texture after thawing.

Some studies reported that greater motor activity, a consequence of the rearing system, is one of the main factors affecting tenderness (Castellini et al., 2002b; Chen et al., 2013) of chicken meat cuts (Castellini et al., 2002a; Farmer et al., 1997). In contrast, some researches (Fanatico et al., 2005; Wang et al., 2009) found that the free-range system had no effect on meat tenderness of the slow-growing birds; and Husak et al. (2008), in a survey of broilers in retail markets, observed that breast and thigh meat from conventional broilers was tenderer than the same cuts from organic and free-range broilers. Many factors such as diet, age, and pre- and post-slaughter handling may affect meat tenderness, making difficult to conclude the isolated effect of rearing system on meat tenderness (Husak et al., 2008).

A reduction ( $P < 0.001$ ) in myofibrillar fragmentation index and total collagen concentration was verified in breast, drumstick, and thigh samples from the conventional and alternative broilers studied throughout the experiment. The myofibril fragmentation index is the process of breaking myofibrils into smaller segments at the Z-line or nearby during the animal postmortem (Olson et al., 1976). According to previous literature (Koochmaraie et al., 1988; Morgan et al., 1993; Taylor et al., 1995; Watanabe et al., 1996), MFI increases continuously during aging of meat from ruminant animals, for example, but, to our knowledge, there are no standards established for MFI in chicken meat, which makes it difficult to rank the degree of tenderness using MFI. Culler et al. (1978), evaluating the relationship between MFI and characteristics of bovine longissimus muscle, defined meat with an MFI value of 60 as soft; meat with MFI close to 50 as intermediate or slightly soft; and meat with MFI close to 35 as tough. We cannot affirm that these values are really applicable to chicken meat, since chicken meat is clearly tenderer than bovine meat and may present similarly MFI values.

In beef, a reverse relation can be established between MFI and tenderness: higher MFI values mean lower shear force and greater softness (Culler et al., 1978; Koochmaraie et al., 1990). The reduction in MFI value was not expected, based on papers previously published, using meat from other species. Our previous studies using chicken meat (Mello et al., 2016; Mello et al., 2017a; Mello et al., 2017b) show that during aging of breast meat, a reduction of MFI values also occurred. There are few studies in the literature about

MFI of chicken meat, and some of them are not conclusive. More studies are necessary to confirm if the inverse relationship between MFI and shear force observed in studies with meat from other species is also valid for chicken meat.

Collagen is the main determinant of meat texture (Bailey and Light, 1989), and the connective tissue consists mainly of collagen molecules that affect meat tenderness, which depends not only on the quantity but also on the structural stability of the collagen molecules (Ramos and Gomide, 2007; Mello et al., 2017c). Collagen shrinks abruptly when heated to temperatures of at least 65°C (Bailey, 1992), which can cause total tissue shrinkage, resulting in fluid loss and changes in structural properties. If the collagenous matrix does not solubilize during heating, it will form a barrier to tissue breaking during chewing. As described previously, the motor activity developed by chickens with access to grazing, throughout their lives, is one of the main factors that affects meat tenderness (Castellini et al., 2002b; Chen et al., 2013), and the toughness characteristic of meat from these broilers may result from changes in the chemical structure of intramuscular collagen and in the cross-links that stabilize fibers (Nishimura, 2015). Regarding the reduction of total collagen amount during freezing, it is possible that, as during aging, the action of enzymes can promote the breaking of insoluble collagen into soluble fragments, resulting in the reduction of muscle collagen content (Oliveira et al., 1998; Mello et al., 2017a).

## CONCLUSION

Freezing for up to 12 mo, suggested by the meat industry in packaging, affects important characteristics related to the succulence of chicken meat, whose effect could be observed mainly in meat from broilers raised in conventional and organic systems. Freezing time also influences the total collagen amount in chicken meat. Therefore, freezing chicken meat cuts for long periods, regardless of the production system in which the broilers were raised, may interfere with the meat texture during preparation and consumption and, consequently, influence the consumer decision in a next purchase.

## ACKNOWLEDGMENTS

The authors thank the São Paulo Research Foundation (Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP, 2012/10276-0) for financial support.

## REFERENCES

- ABPA - Brazilian Association of Animal Protein. 2016. Annual report 2016. Accessed May. 2017. <http://abpa-br.com.br/setores/avicultura/publicacoes/relatorios-anuais>.
- AOAC. 2005. Official Methods of Analysis. 18th ed. Association of Analytical Chemists, Washington, DC.
- Baumel, J. J., A. S. King, J. E. Breazile, H. E. Evans, and J. C. V. Berge. (eds.) 1993. Pages 779 *Nomina Anatomica Avium*. 2nd rev. ed. Cambridge, Berge.
- Bailey, A. J., and N. D. Light. 1989. Connective tissue in meat and meat products. ed. Elsevier, London, UK.
- Bailey, A. J. 1992. Procter memorial lecture: Collagen - nature's framework in the medical, food and leather industries. J. Soc. Leath. Tech. Ch. 76:111–127.
- Campañone, L. A., V. O. Salvadori, and R. H. Mascheroni. 2001. Weight loss during freezing and storage of unpackaged foods. J. Food Eng. 47:69–79.
- Carroll, C. D., and C. Z. Alvarado. 2008. Comparison of air and immersion chilling on meat quality and shelf life of marinated broiler breast fillets. Poult. Sci. 87:368–372.
- Castellini, C., C. Mugnai, and A. Dal Bosco. 2002a. Effect of organic production system on broiler carcass and meat quality. Meat Sci. 60:219–225.
- Castellini, C., C. Mugnai, and A. Dal Bosco. 2002b. Meat quality of three chicken genotypes reared according to the organic system. Italian J. Food Sci. 14:401–412.
- Chen, X., W. Jiang, H. Z. Tan, G. F. Xu, X. B. Zhang, S. Wei, and X. Q. Wang. 2013. Effects of outdoor access on growth performance, carcass composition, and meat characteristics of broiler chickens. Poult. Sci. 92:435–443.
- Culler, R. D., F. C. Parrish, Jr., G. C. Smith, and H. R. Cross. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43:1177–1180.
- Dabes, A. C. 2001. Propriedades da carne fresca. Revista Nacional da Carne, 25:32–40.
- Demirok, E., G. Veluz, W. V. Stuyvenberg, M. P. Castañeda, A. Byrd, and C. Z. Alvarado. 2013. Quality and safety of broiler meat in various chilling systems. Poult. Sci. 92:1117–1126.
- Fanatico, A. C., L. C. Cavitt, P. B. Pillai, J. L. Emmert, and C. M. Owens. 2005. Evaluation of slow-growing broiler genotypes grown with and without outdoor access: Meat quality. Poult. Sci. 84:1785–1790.
- Fanatico, A. C., P. B. Pillai, L. C. Cavitt, J. L. Emmert, J. F. Meullenet, and C. M. Owens. 2006. Evaluation of slower-growing broiler genotypes grown with and without outdoor access: Sensory attributes. Poult. Sci. 85:337–343.
- Fanatico, A. C., P. B. Pillai, J. L. Emmert, and C. M. Owens. 2007. Meat quality of slow- and fast-growing chicken genotypes fed low nutrient or standard diets and raised indoors or with outdoor access. Poult. Sci. 86:2245–2255.
- Fanatico, A. C., C. M. Owens, and J. L. Emmert. 2009. Organic poultry production in the United States: Broilers. J. Appl. Poult. Res. 18:355–366.
- Farmer, L. J., G. C. Perry, P. D. Lewis, G. R. Nute, J. R. Piggott, and R. L. S. Patterson. 1997. Responses of two genotypes of chicken to the diets and stocking densities of conventional UK and label rouge production systems. II. Sensory attributes. Meat Sci. 47:77–93.
- Gomide, L. A. M., E. M. Ramos, and P. R. Fontes. 2013. A carne como alimento; Propriedades da carne fresca. Page 155 in Ciência e qualidade da carne: fundamentos. ed. UFV - Universidade Federal de Viçosa, Viçosa-MG, Brazil.
- Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum protein by means of the biuret reaction. J. Biol. Chem. 177:751–766.
- Hamm, R. 1961. Biochemistry of meat hydration. Adv. Food Res. 10:355–463.
- Hardy, B., N. Crilly, S. Pendleton, A. Andino, A. Wallis, N. Zhang, and I. Hanning. 2013. Impact of rearing conditions on the microbiological quality of raw retail poultry meat. J. Food Sci. 78. doi: 10.1111/1750-3841.12212.
- Honikel, K. O. 1987. The water binding of meat. Fleischwirtsch. 67:1098–1102.
- Husak, R. L., J. G. Sebranek, and K. Bregendahl. 2008. A survey of commercially available broilers marketed as organic, free-range, and conventional broilers for cooked meat yields, meat composition, and relative value. Poult. Sci. 87:2367–2376.
- Koohmaraie, M., A. S. Babiker, A. L. Schroeder, R. A. Merkel, and T. R. Dutson. 1988. Acceleration of postmortem tenderization in ovine carcasses through activation of Ca<sup>2</sup>-dependent proteases. J. Food Sci. 53:1638–1641.



- Koohmaraie, M., G. Whipple, and J. D. Crouse. 1990. Acceleration of postmortem tenderization in lamb and Brahman cross beef carcasses through infusion of calcium chloride. *J. Anim. Sci.* 68:1268–1278.
- Lawson, M. A. 2004. The role of integrin degradation in post-mortem drip loss in pork. *Meat Sci.* 68:559–566.
- Lyon, C. E., B. G. Lyon, and J. A. Dickens. 1998. Effects of carcass stimulation, deboning time, and marination on color and texture of broiler breast meat. *J. Appl. Poult. Res.* 7:53–60.
- Mello, J. L. M., R. A. Souza, G. C. Paschoalin, F. B. Ferrari, M. P. Berton, A. Giampietro-Ganeco, P. A. Souza, and H. Borba. 2016. Physical and chemical characteristics of spent hen breast meat aged for seven days. *Anim. Prod. Sci.* doi: 10.1071/AN16195.
- Mello, J. L. M., R. A. Souza, F. B. Ferrari, A. Giampietro-Ganeco, P. A. Souza, and H. Borba. 2017a. Effects of aging on characteristics of breast meat from free-range broiler hens at 12 or 70 weeks of age. *Anim. Prod. Sci.* doi: 10.1071/AN16523.
- Mello, J. L. M., R. A. Souza, G. C. Paschoalin, F. B. Ferrari, B. M. Machado, A. Giampietro-Ganeco, P. A. Souza, and H. Borba. 2017b. A comparison of the effects of post-mortem aging on breast meat from Cobb 500 and Hubbard ISA broilers. *Anim. Prod. Sci.* doi: 10.1071/AN16603.
- Mello, J. L. M., A. B. B. Rodrigues, A. Giampietro-Ganeco, F. B. Ferrari, R. A. Souza, P. A. Souza, and H. Borba. 2017c. Characteristics of carcasses and meat from feedlot-finished buffalo and *Bos indicus* (Nelore) bulls. *Anim. Prod. Sci.* doi: 10.1071/AN16556.
- Morgan, J. B., T. L. Wheeler, M. Koohmaraie, J. W. Savell, and J. D. Crouse. 1993. Meat tenderness and the calpain proteolytic system in Longissimus muscle of young bulls and steers. *J. Anim. Sci.* 71:1471–1476.
- Nishimura, T. 2015. Role of extracellular matrix in development of skeletal muscle and postmortem aging of meat. *Meat Sci.* 109:48–55.
- Oliveira, L. B., G. J. D. Soares, and P. L. Antunes. 1998. Influence of the maturation of bovine meat in the solubility of the collagen and weight losses for cooking. *Rev. Bras. Agroci.* 4:166–171.
- Oliveira, F. R., C. A. Boari, A. V. Pires, J. C. Mognato, R. M. S. Carvalho, M. A. Santos, and C. C. Mattioli. 2015. Pre slaughter fasting and free-range broilers meat quality. *Rev. Bras. Saúde Prod. Anim.* 16:667–677.
- Olson, D. G., F. C. Parrish, Jr., and M. H. Stromer. 1976. Myofibril fragmentation and shear resistance of three bovine muscles during postmortem storage. *J. Food Sci.* 41:1036–1041.
- Purchas, R. W. 1990. An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. *Meat Sci.* 27:129–140.
- Ramos, E. M., and L. A. M. Gomide. 2007. Avaliação de carnes anormais: Condições PSE e DFD. Pages 531–575 in *Avaliação da qualidade de carnes: Fundamentos e metodologias*. E. M. Ramos, and L. A. M. Gomide. eds. UFV - Universidade Federal de Viçosa, Viçosa-MG.
- SAS Institute Inc. 2002–2003. 'SAS Version 9.1.' SAS Institute Inc., Cary, NC.
- Taylor, R. G., G. H. Geesink, V. F. Thompson, M. Koohmaraie, and D. E. Goll. 1995. Is Z-disk degradation responsible for post-mortem tenderization? *J. Anim. Sci.* 73:1351–1367.
- Wang, K. H., S. R. Shi, T. C. Dou, and H. J. Sun. 2009. Effect of a free-range raising system on growth performance, carcass yield, and meat quality of slow-growing chicken. *Poult. Sci.* 88:2219–2223.
- Watanabe, A., C. C. Daly, and C. E. Devine. 1996. The effects of the ultimate pH of meat on tenderness changes during ageing. *Meat Sci.* 42:67–78.
- Zhang, S. X., M. M. Farouk, O. A. Young, K. J. Wieliczko, and C. Podmore. 2005. Functional stability of frozen normal and high pH beef. *Meat Sci.* 69:765–772.