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USAGE OF HISTOLOGICAL AND RHEOLOGICAL TECHNIQUES IN ASSESSMENT AND PREDICTION OF MEAT TEXTURAL PROPERTIES

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Abstract

The research carried on a comparative assessment of chicken meat texture using both histological and instrumental – rheological techniques, in order to find out certain correlations between the methods and the possibility to predict one in relation with another. Therefore, 50 samples of Pectoralis major and Pectoralis minor muscles, issued from 50 individuals of Cobb-500 chicken broilers were submitted to paraffin inclusion technique followed by microscopic measurements (Motic Image 3+ software after image acquiring at 400 x magnification), respectively to rheological analysis, using a common Adams consistometer and a Perten Instruments texturometer with Warner-Bratzler shear chamber. The histometric values in Pectoralis minor muscles oscillated between 25.18 and 44.37 µm thickness of myocites, a muscle density of 579-683 fibers/sqmm of muscle and a proportion of pure muscular tissue of 62.51% vs. 37.49% connective elements. In Pectoralis major muscles, the proportion of pure muscular tissue reached 59.83% while the connective compounds represented 40.17%, which corresponded to a density of 405-669 fibers/sqmm of muscle and to a myocites thickness within the 28.63-47.19 µm limits. These findings were consistent and highly positive correlated (0.89) with the Warner-Bratzler shear force values (47.10 Newtons in Pectoralis major and 42.63 Newtons in Pectoralis minor muscles) or moderate negatively correlated (-0.53) with the Adams consistency (1,8 Adams units in M. Pectoralis minor and 1,2 Adams units in M. Pectoralis major). Therefore, shear force instrumental assessments could be used as predictors for the histological properties of the meat, including for the tissual composition of muscles (pure muscular vs. connective tissue participation in meat structure).

Key words*: COBB-500, myocytes, thickness, density, shear force, texture.*

INTRODUCTION

Textural properties (tenderness, juiciness) were always considered key point quality and consumer acceptance indicators, because they influence directly the sensorial and technological traits of the meat (Schilling et al., 2003; Zhuang et al., 2007). It was reported that meat texture, especially in poultry, varies due to several influential factors, such as: genotype, due to higher firmness and lower tenderness in pure breeds and slow growing broilers (Wattanachant et al., 2004); selection against certain physical properties of the meat, such as the ultimate pH value (Alnahhas et al., 2015); age at slaughter (Coro et al., 2002); type of culling prior to slaughter and of post-mortem electrical stimulation (Froning and Uijttenboogaart, 1998); farming system, i.e. conventional vs. organic (Grashorn and Serini, 2006); type of applied heat and pressure treatments onto meat (Zamri et al., 2006; Del Omo et al., 2010; Kruk et al., 2011); certain abnormal meat rheological conditions (pale, soft exudative-PSE; dry, firm dark meat–DFD, wooden breast condition-WBC, white stripping-WS) (Barbut et al., 2005; Zhang and Barbut, 2005; Chatterjee et al., 2016; Sanchez et al., 2016); meat chemical composition, including collagen levels (Chumngoen and Tan, 2015). Also, meat texture is directly related to live development of animals and, subsequently, to carcass weight, because the growing process influences both the size of myocytes as well as the ratios between pure muscular and connective tissues in both domestic and wild animal species (Żochowska et al., 2005;Petracci and Cavani, 2012). Muscle ultrastructure, assessed through conventional histometric techniques was longtime used as predictor of meat texture (Stanley and Swatland, 1976), along with sensorial descriptive panel testing (Lyon and Lyon, 2000) and with several objective instrumental methods, such as needle Instron puncture, Allo–Kramer, Warner–Bratzler and razor blade shears tests (Cavitt et al., 2005; Xiong et al., 2006; Luckett et al., 2014). Within this conjuncture, we aimed to test two methods (conventional cyto-histometry and Warner Bratzler shear test) in order to identify the correlations between the results and to find out if both tests could be used together to better predict poultry texture traits, as well as the tissual composition of muscles.

MATERIALS AND METHODS

Fifty samples of *Pectoralis major* and *Pectoralis minor* muscles, issued from 50 individuals of Cobb-500 chicken broilers (conventional farming system, slaughtered at 42 days) were submitted to shaping in 1.5 x 1.5 x 0.3 cm (Width x Length X Thickness) pieces and included in histological processing cassettes. The remnants of the samples were shaped, along the muscle fibres growth direction, in cubic blocks of 1.5 x 1.5 x 1.5 cm (Width x Length X Thickness) and kept in refrigerator throughout 24 hours, in order to allow maturation, then they were submitted to textural instrumental analysis, (Zhuang and Savage, 2009), using a common Adams consistometer to test deformation and a Perten Instruments TVT 7600 texture analyzer equipped with Warner-Bratzler accesories (Steffe, 1996).

The Adams consistometer was equipped with a dial with concentric circles, graded from 0 (origin) to 10, the gap between two consecutive circle measuring 1 cm, sub-graded in mm. Every cm on the scale represents one Adams unit. Each meat sample was placed in the origin spot then a weight of 500 g was added on top. In relation with the meat texture characteristics, the sample flattened and extended more or less on the dial, as well on the scale. The extension was measured on the consistometer scale and was expressed in Adams units with one decimal. Therefore, as the meat is more tender, the extension on the consistometer is wider. Two samples were tested for each muscle; therefore 100 repetitions were run in order to acquire Adams deformation data.

The Pertentexture analyser was equipped with a straight Warner Bratzler blade and with the appropriate rig - heavy duty stand - to test the cutting strength in a single cycle compression mode. Prior to repeated testing, the probe was accordingly calibrated for cutting parameters (starting distance from sample 5 mm; compression 25 mm; initial and testing speed 2 mm/s; trigger force 5 g, data rate 200 pps). The acquired force curves during tests were recorded through the dedicated software (TexCalc 4.0.2.) and the maximum peak force (Newtons) was considered as the shear force necessary to cut the meat sample. Two samples were tested for each muscle; therefore 100 repetitions were run in order to acquire cutting strength data.

The samples prepared for histology processing were immersed in fixation bath (formaldehyde 10%, at 4°C, throughout 30 days).Then, they were submitted to the paraffin infiltration technique using a spin tissue processor - histology line - THERMOSCIENTIFIC STP-120-2(Pappas, 1994) and following a 3 stages protocol: dehydration in 5 consecutive ethyl alcohol baths (70% ethanol, 1 hour; 95% ethanol, 1 hour; 1st absolute ethanol, 1 hour; 2nd absolute ethanol 1,5 hours; 3nd absolute ethanol, 1.5 hours; 4th absolute ethanol, 2 hours); immersion in clearing agent (1st Xylene bath, 1 hour; 2nd Xylene bath, 1 hour); paraffin impregnation (1st paraffin bath at 58°C 1 hour; 2nd paraffin bath at 58°C, 1 hour).

The resulted samples were transferred into stainless molds, accompanied by an appropriate amount of paraffin, at 58°C, then they were cooled down. The resulted solidified blocks were submitted to micrometric cutting using a rotary automatic microtome - histology line - THERMOSCIENTIFIC HM355S adjusted to 5μm step increment. The resulted slices were mounted in groups of 3 pieces on cleaned histological glass slides. These slides were introduced then in a thermos-regulated oven at 65°C, throughout 20 minutes in order to induce slices bonding on the glass.

Slices were then introduced into a trichromic Masson staining protocol, using an automatic tissue stainer - histology line - Varistain Gemini AS – THERMOSCIENTIFIC. The procedure comprised 16 steps: deparaffinizing and rehydration using 3 successive baths of absolute, 95% and 70% ethanol; washing in distilled water; re-fix in Bouin's solution at 56°C, 1 hour; rinsing in tap water, 5-10 minutes; staining in Weigert's iron hematoxylin solution, for 10 minutes; rinsing in tap water, 10 minutes; washing in distilled water, 5 minutes; stain in Biebrich scarlet-acid fuchs in solution, 10-15 minutes; washing in distilled water, 5 minutes; immersing in phosphomolybdic-phosphotungstic acid solution, 10-15 minutes; transfer and staining without rinse into aniline blue solution, 5-10 minutes; rinsing briefly in distilled water; immersing in 1% acetic acid solution for 2-5 minutes; washing in distilled water, 2-5 minutes; dehydrating quickly through 95% ethyl alcohol, absolute ethyl alcohol clearing in xylene; mounting square microscopic slides over the stained sample and sealing with resinous reagent.

The resulting stained and fixed smears were analyzed by microscopic measurements (Motic M230 with camera, calibrated with the default objective micrometric scale for 10 x 10 and 10 x 40 ocular x objective associations) and computations (Motic Image 3+ software after image acquiring at 100X and 400X magnify-cation factors) to assess myocytes and 1st order muscular fascicles diameters (μm) and cross section areas (sqμm).

Two hundred and forty-five myocytes and eight 1st order muscular fascicles were analyzed in each muscle.

Myocytes density (number of muscle cells per sqmm of muscle) and proportion of main tissue categories (% pure muscular tissue and % connective tissue) were also calculated. Muscle cells density was obtained using the relation (Radu-Rusu et al., 2007):

$$\text{Myocytes density} = \frac{\text{myocytes amount in MFI x 1000000}}{\text{cross section area of MFI (sq}\mu\text{m)}},$$

where:

MFI = 1st order muscle fascicle

1000000 = multiplication factor (1 sqmm=1000000 μm)

The proportions of pure muscular and connec-tive tissues in muscle structure were calculated using the relation (Radu-Rusu et al., 2007):

$$P_{MT}\,(\%) = \frac{\Sigma \text{ myocytes cross section areas (sq}\mu\text{m) in MFI}}{\text{cross section area of MFI (sq}\mu\text{m)}}$$

$$P_{CT}\,(\%) = 100 - P_{MT}\,(\%)$$

The acquired data were statistically processed to obtain the main descriptors (mean, standard deviation, coefficient of variation - CV%) and running of comparisons between the used methods (one-way ANOVA), as well as to assess the correlation level between the results, using Graphpad Prism 8.0 for Windows software, in accordance with the appropriate methodology for animal science experiments (Kaps and Lamberson, 2014).

RESULTS AND DISCUSSIONS

Breast myocytesdimensional properties are presented in Table 1, as a comparative analysis between *Pectoralis major* and *Pectoralis minor* muscles.

In *Pectoralis major* muscle (superficial pectoral in breast mass), cells thickness varied between 28.36 μm and 47.19 μm, resulting an average diameter of 37.31±0.28 μm. Hence the coefficient of variation was calculated at 11.72%, the homogeneity of the analyzed trait could be considered low.

Table 1. Dimensional features of myocytes in breast meat

Trait	M	Mean	±SME	CV%	Min.	Max.
Thickness (μm)	PM	37.31	0.28	11.72	28.63	47.19
	Pm	36.22	0.25	10.90	25.18	44.37
ANOVA:	PM vs. Pm (distinct significant): 0.001 <*P*(0.003) < 0.01					
Cross section area (sqμm)	PM	1580.22	11.44	11.33	1157.55	1996.14
	Pm	1537.43	12.27	12.49	1065.11	1987.78
ANOVA:	PM vs. Pm (significant): 0.01 <*P*(0.011) < 0.05					

M = muscle, where PM = Pectoralis major, Pm=Pectoralis minor.
SME = Standard error of the mean
CV% = coefficient of variation

Myocytes in *Pectoralis minor* samples (profound pectoral in breast mass), had diameters comprised within the 25.18 μm and 44.37 μm, therefore an average thickness of 36.22±0.25 μm, while the variation was situated, as well, above the 10% homogeneity threshold. The data we found is comparable with the results reported by MacRae et al., 2007, (38.6 μm – 41.6 μm) in a study analyzing the muscle fibers characteristics in the pectorals of three genetic strains of broilers' genitors. The difference of 1.09 μm (3%) between the cells thickness in the two muscles composing the breast meat was distinct significant ($p < 0.01$).

Average thickness values led to a similar distribution of myocytes cross-section areas when the two muscles were compared. Thus, in *Pectoralis major,* the average cross-section area (1580.22±11.44 sqμm) was significantly 2.8% higher than the one measured in *Pectoralis minor* samples (1537.43±12.27 sqμm) ($p<0.05$). All the values measured within the 1157.55-1996.14 sqμm, respectively between 1065.11-1987.78 sqμm intervals presented low homogeneity (coefficient of variation above 10%).Our results revealed lower cross-section areas that the ones reported in other studies run on same muscles (Berri et al., 2007; Petracci et al., 2013; DalleZotte et al., 2017).

Muscle fibers density was higher in the *Pectoralis minor* samples (624.00±14.62 myocites/sqmm) than in the *Pectoralis major* ones (551.86±33.81 myocites/sqmm), an expected fact, hence the fibers are thicker in *Pectoralis major* (Table 2). The differences between means did not pass the α 0.05 significance threshold.

Table 2. Myocytes density and tissue proportions in breast meat

Trait	M	Mean	±SME	CV%	Min.	Max.
Density (myocites /sqmm)	PM	551.86	33.81	16.21	405.00	669.00
	Pm	624.00	14.62	6.20	579.00	683.00
ANOVA:	PM vs. Pm (not significant): 0.05 <*P* (0.073)					
Striate muscular tissue (%)	PM	59.83	1.15	5.10	56.00	64.20
	Pm	62.51	1.41	5.96	58.00	67.10
ANOVA:	PM vs. Pm (not significant): 0.05 <*P* (0.167)					
Connective tissue (%)	PM	40.17	1.15	7.60	35.80	44.00
	Pm	37.49	1.41	9.94	32.90	42.00
ANOVA:	PM vs. Pm (not significant): 0.05<*P*(0.167)					

M = muscle, where PM = Pectoralis major, Pm=Pectoralis minor.
SME = Standard error of the mean
CV% = coefficient of variation

These findings were correlated with the main tissual categories in muscles, knowing that the thinner and more densified are the muscle cells, the less room remains for connective tissue in that particular muscle (Petracci et al., 2013). Thus, pure muscle tissue participation reached 59.83±1.15% in *Pectoralis major* and 62.51±1.41% in *Pectoralis minor* while the connective tissue was higher in the former muscle (40.17±1.15%) than in the latter one (37.49±1.41%). Although these differences were not found as statistically significant ($p>0.05$), the histological findings suggest that the meat of *Pectoralis minor* muscles would have a better texture, with thinner cells and less connective tissue, therefore a better sensorial quality.

The textural instrumental analysis (table 3) on both muscles revealed, as indicated also by the histological findings, a 10.49% lower cutting strength in the *Pectoralis minor* samples (42.63±0.21 Newtons), compared with the *Pectoralis major ones* (47.10±0.33 Newtons). Significant differences were calculated between the two muscles ($p<0.05$). The findings were homogenous, if the lower values of coefficients of variation are considered (5.01-6.97%).

Table 3. Texture instrumental analysis of breast meat samples

Trait	M	Mean	±SME	CV%	Min.	Max.
Shear Force (Newtons)	PM	47.10	0.33	6.97	43.18	52.90
	Pm	42.63	0.21	5.01	38.82	44.70
ANOVA:	PM vs. Pm (significant): 0.01<*P* (0.028) < 0.05					
Adams consistometry (A.U.)	PM	1.2	0.01	6.27	1.1	1.3
	Pm	1.8	0.01	9.34	1.6	2.1
ANOVA:	PM vs. Pm (highly significant): *P* (5.31×10^{-5}) < 0.001					

M = muscle, where PM = Pectoralis major, Pm=Pectoralis minor.
SME = Standard error of the mean
CV% = coefficient of variation

Meat extension on the Adams consistometer revealed values higher in *Pectoralis minor* (1.8±0.01 Adams units) and lower in *Pectoralis major* samples (1.2±0.01 Adams units), suggesting a better tenderness in *Pectoralis minor* and a better firmness in *Pectoralis major* ($p<0.001$).

The acquired data related to cutting strength and firmness are consistent to those reported in other similar studies (Xiong et al., 2006; Zhuang and Savage, 2009; Chatterjee et al., 2016).

Certain histological traits were highly correlated with the textural ones: strong positive correlations between the myocytes thickness and shear force (cutting strength) (r=+0.89 in *Pectoralis major* and r=+0.95 in *Petoralis minor*) (Table 4).

Table 4. Correlations between hitsometrical and rheological traits

Muscle	Trait	Shear Force	Adams Consistometry
Pectoralis major	Myocites thickness	r=+0.89	r=-0.53
	Myocites density	r=-0.68	r=-0.29
	Muscular tissue	r=-0.88	r=+0.14
	Connective tissue	r=+0.88	r=-0.14
Pectoralis minor	Myocites thickness	r=+0.95	r=-0.11
	Myocites density	r=-0.86	r=-0.17
	Muscular tissue	r=-0.43	r=+0.05
	Connective tissue	r=+0.43	r=-0.05

Also, the shear force was intense positive (+0.88) or medium positive correlated with the connective tissue proportion. In fact, the higher the connective tissue proportion, the stronger the correlation with the shear force value, knowing that the connective tissue (either specific cells, such as adipocytes or tenocytes, either the extracellular matrix, particularly the collagen) negatively affects the meat textural characteristics, assessed both instrumentally and sensorial (Roy et al., 2006; Nishimura 2010).
On the contrary, shear force was negatively and intense correlated with myocytes density and with the muscular tissue proportion in the analyzed muscles, suggesting that higher the amount of muscle cells, lower will be the force necessary to cut this particular sample.
Adams consistometry revealed poor to medium negative correlations with most of the histological traits, except with the muscular tissue proportion. Therefore, the sample extensibility (deformation) is higher when less connective tissue is present in the sample or sample firmness is higher as this particular tissual category increases its participation in muscle formation.

CONCLUSIONS

Pectoralis minor muscles had thinner fibers, higher myocytes density and higher proportion of pure muscular tissue, compared to *Pectoralis major* muscles.
The instrumental texture analysis indicated lower shear force and better extensibility in *Pectoralis minor*, suggesting better tenderness, versus the *Pectoralis major* findings.
Acquired data confirm that certain textural descriptors of the chicken meat (especially shear force) are highly and positively correlated with some of the meat histological characteristics (especially myocytes dimensional features). Therefore, one trait, once measured, could predict the other one with which is highly correlated.
As follow-up, it is indicated to enlarge the panel of textural descriptors to be measured instrumentally and sensorial, as well, in order to correlate the human consumer perception for those descriptors with the textural instrumental or histological findings. In this particular situation, our study reveals better tenderness and masticability of *Pectoralis minor* muscles, but this statement remains to be confirmed by the sensorial analysis.

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