Session VIII Muscle Biochemistry and Meat Quality

Session VIII.a Pages

N/A= The short paper is not available, either because the authors reported to the editor they do not wish to publish their work, or because the authors did not respond to the editor's invitation to publish.

WATER-HOLDING CAPACITY AND PROTEIN DENATURATION IN BROILER BREAST MEAT

Brian Bowker[1*] and Hong Zhuang[1]

[1]USDA-ARS, Quality & Safety Assessment Research Unit, Athens, Georgia, USA

*brian.bowker@ars.usda.gov

Abstract – The aim of this study was to determine the relationship between water-holding capacity (WHC) and protein denaturation in broiler breast meat. Breast fillets were collected at 2 h postmortem and segregated into low- and high-WHC groups based on pH and color. Protein solubility was measured at 6 and 24 h postmortem as an indicator of protein denaturation. Brine uptake and drip loss were measured for WHC. High-WHC fillets had greater brine uptake at 6 and 24 h postmortem and less accumulated drip loss after 2 and 7 days compared to low-WHC fillets. Brine uptake increased with postmortem time of sampling. Myofibrillar protein solubility decreased with postmortem time but was not different between low- and high-WHC fillets. Sarcoplasmic protein solubility increased with postmortem time and was greater in high-WHC fillets. Further analysis showed that brine uptake and drip loss were correlated to the relative abundance of glycogen phosphorylase in both protein fractions, and increased glycogen phosphorylase denaturation was observed in low-WHC fillets. Data suggest that the denaturation of glycogen phosphorylase onto myofibrils may influence WHC but that inherent differences in myofibrillar protein denaturation are not the predominant source of WHC variation in broiler breast fillets.

I. INTRODUCTION

Broiler breast muscle, which is comprised of nearly 100% fast-twitch glycolytic muscle fibers, is highly susceptible to developing inferior water-holding capacity (WHC). The ability of meat to bind water is a complex trait that is influenced by structural and biochemical changes that occur during the transformation of muscle to meat. Muscle pH and protein denaturation are considered the main determinants of WHC in meat [1]. Protein solubility and extractability are often used as measures of protein denaturation within meat. In pork and turkey muscle, low WHC is often associated with excessive postmortem protein denaturation, particularly that of myosin [2, 3]. The underlying mechanisms that control WHC in pale broiler meat are not as well-established. Some reports have shown that myofibrillar proteins from chicken breast muscle are resistant to denaturation [4] and that pale and normal colored fillets have similar protein solubilities [5]. Others have observed lower salt-soluble protein extractability in pale breast meat [6] and increased sarcoplasmic protein denaturation in fillets incubated at elevated temperatures [7]. In broiler breast meat, the effect of postmortem time on the relationship between WHC and protein denaturation is unknown. The objective of this study was to determine muscle protein solubility in broiler breast fillets with widely varying WHC at different times postmortem.

II. MATERIALS AND METHODS

Deboned butterfly breast fillets (n=72) were obtained from a commercial processing line and separated into two groups (low-WHC, high-WHC) based on muscle pH and color values (L*a*b*). At 6 and 24 h postmortem, muscle samples from each butterfly fillet were taken for measurement of water-holding capacity and protein solubility. Brine uptake (%) was measured by homogenization and centrifugation in 0.6 M NaCl buffer [8]. Accumulated drip loss (%) was measured after storage for 2 and 7 days postmortem at 4°C. Protein solubility was measured in 0.25 mM potassium phosphate (pH 7.2) buffer (sarcoplasmic protein solubility) and in 0.1 M potassium phosphate/1.1 M KI buffer (total protein solubility). Myofibrillar solubility was calculated from the difference between total and sarcoplasmic protein solubility.

Table 1. Meat quality measurements (lsmeans) of low-WHC and high-WHC broiler breast fillets.

Measurement:	Low-WHC	High-WHC	SEM
pH 4h	5.99[b]	6.17[a]	0.03
pH 24h	5.83[b]	6.20[a]	0.03
L*	61.3[a]	46.3[b]	0.4
a*	0.4[b]	0.9[a]	0.1
b*	13.0[a]	9.2[b]	0.3

[ab] LSmeans with different letters differ (p<0.05)

At 24 h postmortem, myofibrillar and sarcoplasmic protein fractions were separated by subcellular fractionation [3] in buffer containing 50 mM KCl, 20 mM Tris, pH 7.0, 2 mM EDTA, 4 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 0.1 mM PMSF, and 1% (v/v) Triton X-100, and in rigor buffer (75 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$ 2 mM EGTA, pH 7.0). Protein fractions were loaded onto 4-20% Tris-glycine gels for SDS-PAGE analysis and for transfer to PVDF membrane to detect glycogen phosphorylase in the protein fractions by western blotting. Data were analyzed using PROC MIXED (SAS v. 9.2) models with group (low-WHC, high-WHC) and postmortem time as fixed effects and carcass as a random effect.

III. RESULTS AND DISCUSSION

Selecting broiler breast fillets by color resulted in two distinct groups of samples with regards to meat quality attributes. Expectedly, the pale (low-WHC) fillets had lower pH and higher L* values than dark (high-WHC) fillets (Table 1). At 6 and 24 h postmortem, brine uptake in high-WHC fillets was approximately 3- to 4-fold greater than in low-WHC fillets (Fig. 1).

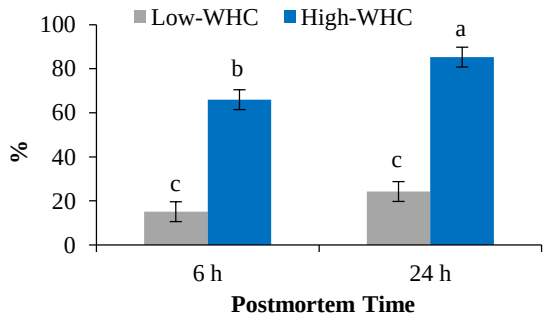

Fig. 1. Brine uptake (%) of broiler breast fillets at 6 and 24 h postmortem.

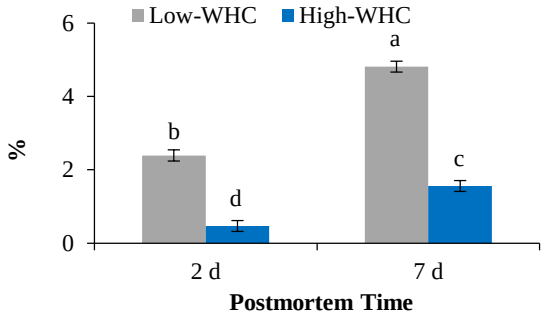

Fig. 2. Accumulated drip loss (%) of broiler breast fillets after 2 and 7 days.

From 6 to 24 h postmortem, brine uptake increased 9% in low-WHC fillets and 20% in high-WHC fillets. Drip loss accumulations were greater in low-WHC fillets after both 2 and 7 days of refrigerated storage (Fig. 2).

Protein solubility was used as an indicator of postmortem protein denaturation within the breast fillets. Myofibrillar protein solubility was similar between low- and high-WHC breast fillets at both 6 and 24 h postmortem (Table 2). Thus, despite the widely divergent WHC attributes between the pale and dark fillets, the overall degree of myofibrillar protein denaturation was not different between the two groups of fillets used in this study. Furthermore, correlation analysis indicated that myofibrillar protein solubility was not related to brine uptake or drip loss (Table 3). These findings suggest that myosin denaturation is not the primary determinant of WHC differences in pale and dark broiler breast fillets and support the idea that myosin from chicken breast meat is resistant to denaturation [4]. Similarly, Van Laack [5] observed only minor differences in total protein solubility between pale and normal colored breast fillets.

Table 2. Protein solubility measurements (lsmeans) of low-WHC and high-WHC broiler breast fillets.

Measurement:		Low-WHC	High-WHC	SEM
Myofibrillar[1]	6 h	121.3[ab]	121.9[a]	2.8
	24 h	114.3[ab]	113.9[b]	
Sarcoplasmic[1]	6 h	79.3[c]	82.7[b]	1.8
	24 h	84.2[b]	88.0[a]	

[ab] LSmeans with different letters within a measurement differ (p<0.05)

[1] Solubility measurements expressed as mg protein/g tissue

Table 3. Correlation coefficients (r) between WHC and protein solubility measurements.

		Brine Uptake		Drip Loss	
Protein Solubility:		6 h	24 h	2 d	7 d
Myofibrillar	6 h	0.13	0.15	-0.14	-0.01
	24 h	0.03	0.09	-0.12	-0.03
Sarcoplasmic	6 h	0.08	0.13	-0.33*	-0.17
	24 h	0.06	0.08	-0.31*	-0.11

* P<0.01

The denaturation of sarcoplasmic proteins is also thought to play a role in determining WHC in meat [9]. In the current study, sarcoplasmic protein solubility was slightly greater in high-WHC breast fillets at both 6 and 24 h postmortem (Table 2). Similar differences in sarcoplasmic protein solubility have also been observed between pale and normal colored broiler breast meat [5].

Other than being weakly related to drip loss accumulation at 2 days, overall sarcoplasmic protein solubility was not correlated to WHC parameters (Table 3). Further analysis showed that within the sarcoplasmic protein fraction of the muscle, glycogen phosphorylase seemed to play a key role in determining WHC. In low-WHC fillets, SDS-PAGE analysis (Fig. 3) indicated that the relative abundance of glycogen phosphorylase was decreased in the sarcoplasmic protein fraction and increased in the myofibrillar fraction compared to high-WHC fillets (Table 4).

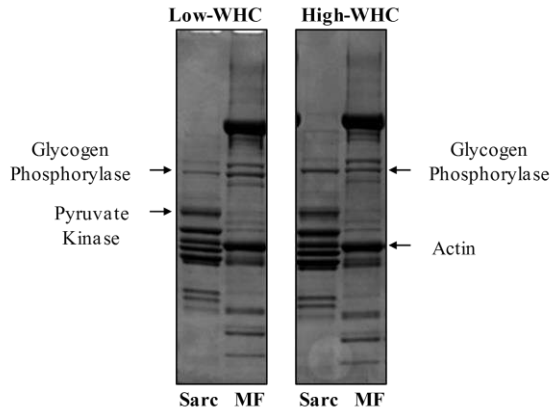

Fig. 3. SDS-PAGE of sarcoplasmic and myofibrillar protein fractions from broiler breast fillets at 24 h postmortem.

Table 4. Relative abundance (lsmeans) of glycogen phosphorylase in myofibrillar and sarcoplasmic protein fractions from broiler breast fillets.

Protein Fraction:	Low-WHC	High-WHC	SEM
Myofibrillar[1]	0.27[a]	0.15[b]	0.01
Sarcoplasmic[2]	0.28[b]	0.52[a]	0.03

[ab] LSmeans with different letters differ (p<0.05)
[1] Glycogen phosphorylase band intensity expressed relative to actin band intensity.
[2] Glycogen phosphorylase band intensity expressed relative to pyruvate kinase band intensity.

This phenomenon was likely due to the early postmortem pH and temperature conditions of the low-WHC fillets causing glycogen phosphorylase to denature and precipitate onto the myofibrils. A similar shift between the sarcoplasmic and myofibrillar protein fractions due to glycogen phosphorylase denaturation has been shown in both turkey and pork [3, 10]. In chicken, incubation of breast muscles at 40°C has been found to induce glycogen phosphorylase denaturation, cause similar shifts in SDS-PAGE banding patterns, and result in higher drip loss [7]. In the current study, the relative abundance of glycogen phosphorylase in both the myofibrillar and sarcoplasmic protein fractions of the breast fillets was strongly related to brine uptake and drip loss measurements (Table 5).

Table 5. Correlation coefficients (r) between WHC and relative abundance of glycogen phosphorylase in myofibrillar and sarcoplasmic protein fractions.

		Brine Uptake		Drip Loss	
Protein Fraction:		6 h	24 h	2 d	7 d
GP-mf[1]	6 h	-0.78***	-0.76***	0.63**	0.66**
	24 h	-0.80***	-0.78***	0.68**	0.72**
GP-sarc[2]	6 h	0.79***	0.80***	-0.64*	-0.67*
	24 h	0.69**	0.70**	-0.58*	-0.61*

* P<0.01, ** P<0.001, *** P<0.0001
[1] Glycogen phosphorylase in myofibrillar protein fraction
[2] Glycogen phosphorylase in sarcoplasmic protein fraction

Aging from 6 to 24 h postmortem, influenced both WHC and overall protein solubility in breast fillets. In general, brine uptake and sarcoplasmic protein solubility increased with aging from 6 to 24 h postmortem and myofibrillar protein solubility decreased (Fig. 1 and Table 2). The relationships between WHC and overall protein solubility measurements

were not influenced by postmortem time of sampling and measurement (Table 3). Correlations between WHC measurements and glycogen phosphorylase abundance were similar at 6 and 24 h postmortem (Table 5).

IV. CONCLUSION

In conclusion, this study demonstrates that overall protein solubility measurements are not closely related to low WHC in broiler breast meat, regardless of postmortem time of measurement (6 or 24 h). These data suggest that myosin denaturation, as measured by myofibrillar protein solubility, is not a distinguishing factor in WHC between pale and dark fillets. Increased glycogen phosphorylase denaturation was observed in fillets with low WHC. This study suggests that while the denaturation of glycogen phosphorylase and its precipitation onto myofibrils may not affect overall myofibrillar protein solubility, it may have a direct impact on WHC attributes. Based on the results of this study, it can be hypothesized that the precipitation of denatured glycogen phosphorylase onto myofilaments may alter their surface interactions with water and lower WHC in broiler breast meat. Further research is needed, however, to determine the mechanism by which sarcoplasmic protein denaturation influences WHC in breast fillets.

V. ACKNOWLEDGEMENTS

The authors would like to thank Elizabeth Barton (USDA-ARS) and Andrew Ross (USDS-ARS) for their technical support in completing this research.

VI. REFERENCES

1. Offer, G. & Knight, P. (1988). The structural basis of water-holding in meat. Part 2: Drip losses. In R. A. Lawrie, Developments in meat science (pp 173-241). London: Elsevier Applied Science.
2. Warner, R. D., Kauffman, R. G. & Greaser, M. L. (1997). Muscle protein changes postmortem in relation to pork quality traits. Meat Science 45:339-352.
3. Pietrzak, M., Greaser, M. L. & Sosnicki, A. A. (1997). Effect of rapid rigor mortis processes on protein functionality in *Pectoralis major* muscle of domestic turkeys. Journal of Animal Science 75:2106-2116.
4. Van Laack, R. L. J. M. & Lane, J. L. (2000). Denaturation of myofibrillar proteins from chicken as affected by pH, temperature, and adenosine triphosphate concentration. Poultry Science 79:105-109.
5. Van Laack, R. L. J. M., Liu, C. H., Smith, M. O. & Loveday, H. D. (2000). Characteristics of pale, soft, exudative broiler breast meat. Poultry Science 79:1057-1061.
6. Barbut, S., Zhang, L. & Marcone, M. (2005). Effects of pale, normal, and dark chicken breast meat on microstructure, extractable proteins, and cooking of marinated fillets. Poultry Science 84:797-802.
7. Zhu, X., Ruusunen, M., Gusella, M., Zhou, G. & Puolanne, E. (2011). High postmortem temperature combined with rapid glycolysis induces phosphorylase denaturation and produces pale and exudative characteristics in broiler *Pectoralis major* muscles. Meat Science 89:181-188.
8. Zhuang, H. & Savage, E. M. (2012). Postmortem aging and freezing and thawing storage enhance ability of early deboned chicken pectoralis major muscle to hold added salt water. Poultry Science 91:1203-1209.
9. Puolanne, E. & Halonen, M. (2010). Theoretical aspects of water-holding in meat. Meat Science 86:151-165.
10. Joo, S. T., Kauffman, R. G., Kim, B. C. & Park, G. B. (1999). The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. Meat Science 52:291-297.

ISOLATION AND PURIFICATION OF DECORIN FROM BOVINE SKELETAL MUSCLE AND ITS STRUCTUAL CHANGES UNDER HIGH PRESSURE

Takumi Hosono[1*], Takahito Komoda[1] and Tadayuki Nishiumi[1]

[1] Division of Life and Food Science, Graduate School of Science and Technology, Niigata University, Niigata, Japan

*f13d037e@mail.cc.niigata-u.ac.jp

Abstract - High hydrostatic pressure induces a weakening of intramuscular connective tissue, which is mainly composed of collagen. Decorin, a small proteoglycan, binds to and stabilizes collagen fibrils. It has been suggested that the weakening of intramuscular connective tissue may result from alteration of the decorin-collagen interaction due to structural changes of the decorin molecule. In this study, decorin was isolated and purified from bovine skeletal muscle by successive steps of an extraction with 4 M guanidine hydrochloride, CsCl density gradient ultracentrifugation, DEAE-cellulose ion-exchange chromatography, and Sepharose CL-6B gel filtration. The isolated decorin possessed an average molecular mass of 100 kDa and contained a core protein mass of 48 kDa by SDS-PAGE. The structural changes in decorin from bovine skeletal muscle were investigated by measuring fluorescence spectra under high pressure.

I. INTRODUCTION

The use of high-pressure technology in food processing has steadily increased over the past 10 years. Among products processed using high pressure, the number and variety of meat and meat products have risen dramatically worldwide (1). High pressure is also used for tenderizing meat or accelerating postmortem aging of meat (2-5). High hydrostatic pressure affects actomyosin toughness and background toughness, leading to meat tenderization. The effect of high pressure on background toughness ascribed to connective tissue, is gradually becoming clearer. Ichinoseki *et al.* (6) reported that high pressure did not degrade collagen molecules but dissociated collagen fibrils. Decorin is one of small proteoglycans and binds to and stabilizes collagen fibrils (7-8). It has been suggested that a weakening of intramuscular connective tissue may result from structural changes to decorin, leading to alteration of the decorin-collagen interaction. Komoda *et al.* (9) found a change of the native structure of decorin molecules from bovine articular cartilage under high pressure at 200-400 MPa. It is assumed that structural changes of decorin molecule are induced by high-pressure processing. In this study, decorin was isolated and purified from bovine skeletal muscle by successive steps of an extraction with 4 M guanidine hydrochloride, CsCl density gradient ultracentrifugation, DEAE-cellulose ion-exchange chromatography, and Sepharose CL-6B gel filtration. The structural changes of purified decorin were then analyzed by fluorescence spectra under high pressure.

II. MATERIALS AND METHODS

Longissimus dorsi muscles were dissected from each carcass of two 3-month-old Holstein steers, trimmed to remove all visible external fat and epimysium, and then stored at -30°C. Decorin was extracted and purified according to the method of Nishiumi *et al*. (7) with slight modifications. The muscles were minced finely, homogenized briefly in a Waring blender (Mauda, Japan) with 4 volumes of a solution containing 4 M guanidine hydrochloride, 1 M sodium acetate (pH 6.0), 0.1 M 6-aminohexanoic acid, 1 mM benzamidine hydrochloride, 10 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride, and extracted for 72 h at 4°C with gentle stirring. The supernatant was collected by centrifugation at 24,900 x *g* for 1 h at 4°C.
To separate decorin from other proteins of the muscle, direct dissociative CsCl density gradient ultracentrifugation was employed. The density of the guanidine hydrochloride

extract was adjusted with CsCl to about 1.37 g/mL, and ultracentrifuged (CP-80WX; Hitachi, Japan) at 156,000 x *g* for 48 h at 4°C in 40 PA tubes (Hitachi) on an angle rotor (P50-AT2; Hitachi). After ultracentrifugation, the tubes were divided into four fractions (D1-D4; D1=bottom) according to the CsCl density. The D2 fraction (density=1.43 g/mL) was subjected to further purification. The fraction was extensively dialyzed against 20 mM Tris-acetate buffer (pH 7.0) containing 7 M urea and applied to a DEAE-cellulose column (2.5 x 25 cm; Sigma, U.S.A.) equilibrated with the same buffer at 4°C. After washing the column with the same buffer, decorin were eluted with a linear gradient of 0-0.5 M NaCl in 7 M urea and 20 mM Tris-acetate buffer (pH 7.0) at a rate of 15 mL/h (one fraction, 10 mL). The content of uronic acid in each fraction was determined (10), and fractions containing decorin were pooled. Further purification was performed by Sepharose CL-6B gel filtration. The pooled fraction containing decorin was concentrated to about 10 mL by ultrafiltration, and then applied to a Sepharose CL-6B column (1.5×120 cm; Sigma) equilibrated with 420 mL of 50 mM Tris-acetate buffer (pH 7.0) containing 4 M guanidine hydrochloride. Decorin was eluted with the same buffer at 4°C at a rate of 10 mL/h (one fraction, 5 ml). The content of uronic acid in each fraction was measured and subjected to SDS-PAGE analysis.

SDS-PAGE was carried out on 7.5% polyacrylamide slab gels with a 3.75% stacking gel, according to the method of Laemmli (11). The gels were stained with Coomassie Brilliant Blue R-250 or Silver staining for proteins or Alcian blue for glycosaminoglycans (12).

Fluorescence spectra were obtained using a spectrometer (F-2500; Hitachi) fitted with a high-pressure vessel (PCI-400; Syn Corporation, Japan) and pump (TP-500; Syn Corporation). A decorin solution (0.2 mg/mL in 100 mM Tris-HCl buffer solution (pH 7.3) was subjected to a range of pressures of between 0.1 and 400 MPa at 50 MPa intervals during approximately 10 s, and fluorescence spectra were measured at each pressure 10 min after pressure was achieved. After 400 MPa compression, the decorin solution was decompressed continually at 50 MPa intervals during approximately 10 s. Fluorescence spectra between 300 and 450 nm with excitation at 280 nm were recorded for decorin during the pressurization and depressurization. Changes in the center of the spectral mass (ν) were calculated in accordance with the method of Ruan *et al.* (13),

$$(\nu) = \Sigma \nu i * Fi / \Sigma Fi$$

where νi is the wavenumber and Fi is the fluorescence intensity at νi.

III. RESULTS AND DISCUSSION

The density and uronic acid content of each fraction after centrifugation is shown in Table 1. As the target, decorin was contained in the D2 fraction, in which a small size proteoglycan (about 100 kDa) was shown by SDS-PAGE. The D2 fraction was then applied to DEAE-cellulose ion-exchange chromatography for further purification. Uronic acid-containing materials were eluted as four peaks at fraction numbers (NaCl concentrations) of 17 (0.17 M), 19 (0.20 M) 25 (0.26 M), 32 (0.33 M), respectively (Fig. 1). Among them, fractions 30-40 at 0.30-0.41 M NaCl contained decorin with a molecular mass of 100 kDa (Fig. 2), and were subjected to Sepharose CL-6B gel filtration chromatography. A large proportion of the uronic-acid containing materials was recovered in fractions 22-30, shown as the decorin fraction in Fig. 3.

SDS-PAGE of isolated decorin from bovine skeletal muscle revealed one band with an average mass of 100 kDa (Fig. 4), which was stained with Silver staining. After chondroitinase ABC treatment, a molecular mass of 48 kDa was confirmed, which is the core protein derived from decorin (data not shown). These results suggest that decorin was purified from bovine skeletal muscle. However, further experiments such as electrophoretic separation of the GAG chains and N-terminal amino acid sequencing of the core protein was required.

At present, structural changes of decorin from bovine skeletal muscle are measured by fluorescence spectra under high pressure.

Table 1. Distribution of glycosaminoglycan (GAG) uronic acid on CsCl density gradient ultracentrifugation of 4 M guanidine hydrochloride extract from bovine skeletal muscle

fraction	density (g/mL)	GAG uronic acid (mg/100 g meat)
D1 (bottom)	**1.51**	**28.9**
D2	**1.43**	**3.0**
D3	**1.37**	**2.2**
D4	**1.33**	**2.6**

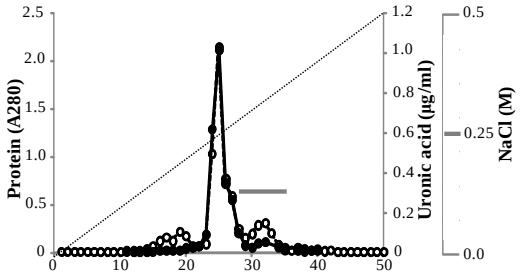

Fig. 1. DEAE-cellulose ion-exchange chromatograph of decorin from bovine skeletal muscle. The absorbance at 280 nm (○) and the uronic acid content of each fraction (●) were determined. Fractions 31-38, indicated by the bar, were combined for further purification.

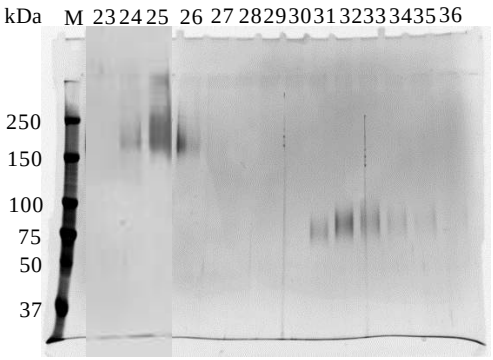

Fig. 2. SDS-PAGE after application of DEAE-cellulose ion-exchange chromatography. The gel was stained with Silver staining. M, molecular weight marker; 23-36, fraction numbers.

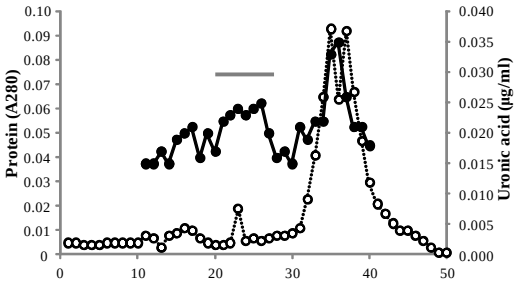

Fig. 3. Sepharose CL-6B gel filtration chromatograph of decorin from bovine skeletal muscle. The absorbance at 280 nm (○) and the uronic acid content of each fraction (●) were determined. Decorin was contained in fractions 22-30, indicated by the bar.

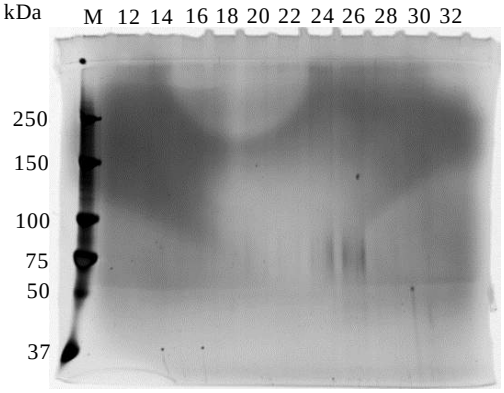

Fig. 4. SDS-PAGE after application of Sepharose CL-6B gel filtration. The gel was stained with Silver staining. M, molecular weight marker; 12-32, fraction numbers.

IV. CONCLUSION

Isolation and purification of decorin from bovine skeletal muscle was carried out by successive steps of an extraction with 4 M guanidine hydrochloride, CsCl density gradient ultracentrifugation, DEAE-cellulose ion-exchange chromatography, and Sepharose CL-6B gel filtration. Purified decorin was characterized and alterations in its molecular structure under high pressure was analyzed.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science (JSPS) to Tadayuki Nishiumi.

REFERENCES

1. Simonin, H., Duranton, F. & de Lambalerie M. (2012). New insight into the high pressure processing of meat and meat products. Comprehensive Reviews in Food Science and Food Safety 11: 285-306.
2. Macfarlane, J. J. (1973). Pre-rigor pressurization of muscle: Effects on pH, shear value and taste panel assessment. Journal of Food Science 38: 294-298.
3. Bouton, P. E., Harris, P. V. & Macfarlane, J. J. (1980). Pressure-heat treatment of meat: Effect of prior aging treatments on shear properties. Journal of Food Science 45: 276-279.
4. Ledward, D. A. (1995). High pressure processing-the potential. In D. A. Ledward, D. E. Johnston, R. G. Earnshaw & A. P. M. Hasting, High pressure processing of foods (pp 1-5). Nottingham: Nottingham University Press.
5. Messens, W., Camp, J. V. & Huyghebaert A. (1997). The use of high pressure to modify the functionality of food proteins. Trends in Food Science and Technology 8: 107-112.
6. Ichinoseki, S., Nishiumi, T. & Suzuki, A. (2006). Tenderizing effect of high hydrostatic pressure on bovine intramuscular connective tissue. Journal of Food Science 71: E276-E281.
7. Nishiumi, T., Fukuda, T. & Nishimura, T. (1997). Isolation and characterization of a small proteoglycan associated with porcine intramuscular connective tissue. Journal of Agricultural and Food Chemistry 45: 2978-2983.
8. Nishimura, T., Futami, E., Wakamatsu, J. & Hattori, A. (2003) Identification and characterization of proteoglycans in bovine neonatal muscle. Animal Science Journal 74: 407-416.
9. Komoda, T., Kim, Y., Suzuki, A. & Nishiumi, T. (2013). A scanning fluorescence spectroscopy of decorin under high pressure. High Pressure Research 33: 336-341.
10. Bitter, T. & Muir, H. M. (1962). A modified uronic acid carbazole reaction. Analytical Biochemistry 4: 330-334.
11. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.
12. Fisher, L. W., Termine, J. D., Dejter, S. W., Jr., Whitson, S.W., Yanagishita, M., Kimura, J. H., Hascall, V. C., Kleinman, H. K., Hassell, J. R. & Nilsson, B. (1983). Proteoglycans of developing bone. The Journal of Biological Chemistry 58: 6588-6594.
13. Ruan, K., Lange, R., Zhou, Y. & Balny C. (1998). Unusual effect of high hydrostatic pressure on basic phospholipase A_2 from venom of *Agkistrodon Halys Pallas*. Biochemical and Biophysical Research Communications 249: 844-848.

Temperament classification affects tenderness of steaks from Simmental x Angus cross steers.

Jane A. Boles[1], Katelyn S. Kohlbeck[1], Michael C. Meyers[2], Katharine A. Perz[1], Jennifer Thomson[1] and Kathleen C. Davis[1]

[1]Department of Animal and Range Sciences, Montana State University, Bozeman, Montana, USA 59717; [2]Department of Sport Science and Physical Education, Idaho State University, Pocatello, Idaho USA 83209.

Abstract – The objective of this study was to determine if temperament impacted growth rate of an animal and tenderness of beef steaks, and could blood lactate be used as an objective measurement of temperament. Simmental x Angus steers were evaluated for exit velocity, blood lactate and docility score (n=154), humanely harvested and tenderness of steaks evaluated (n=30). Steers with a higher ADG resulted in steaks with lower shear force values (P=0.02) than either medium or slow ADG. Steers with high and low blood lactate concentrations resulted in more tender steaks than steaks from steers with medium blood lactate. Steers that exited the chute more quickly tended to result in steaks that had higher shear force values (P=0.06). Results suggest that temperament as measured by exit velocity and blood lactate along with growth rate contribute to variation in tenderness.

I. INTRODUCTION

Variation in meat quality and tenderness is a challenge for the meat industry (1). Many ante and post-mortem factors contribute to this variation including meat pH either rate or extent of decline. Decline of pH postmortem is influenced by numerous factors including temperature, genetics and anti-mortem stress. Apple and co-workers (2) reported increased consumption of glycogen by muscle and altered pH decline postmortem in response to restraint. Stress response results in increased concentrations of epinephrine and cortisol which result in increased gluconeogenesis, proteolysis and increased sensitivity of lipids to lipolytic hormones (3). This response generally leads to increased anaerobic metabolic processes in the muscle and, subsequently, excessive lactic acid in the blood. Curley et al. (4) reported that exit velocity was related to cortisol levels and suggesting the use of exit velocity as a measure of stress during handling.

Café and co-workers (5) evaluated persistent assessments of temperament on productivity, carcass characteristics and meat quality traits (color and tenderness). They found in general, cattle with more excitable temperaments as measured by exit velocities and chute scores had consistently lower feed intakes and slower growth rates, which resulted in smaller carcasses with less fat cover and poorer objective meat quality characteristics. Other researchers also reported decreased growth rates (average daily gain) in animals with more excitable temperaments (6, 7). Along with decreased growth rates, researchers reported that meat from animals with more excitable temperament had higher shear force values (5, 8, 9) and were more likely to produce carcasses that were borderline dark cutters (8). This relationship was seen to be stronger in *bos indicus* breeds than *bos taurus* breeds.

II. MATERIALS AND METHODS

Data was collected in compliance with Montana State University Agriculture Animal Care and Use Committee under the Animal Care approval number 2013-AA02. Angus x Simmental calves (n=154) were evaluated for temperament using chute scores, blood lactate and chute exit velocity measurements. The chute scores, ranging from 1 to 6 were assigned by a single individual at all handling times using the scoring system used by the Beef Improvement Federation (10).

Blood lactate concentration was analyzed with a blood lactate meter (Lactate Pro, Arkray Inc, Minami-Ku, Kyoto, Japan) (mmol/L of lactate). Steers were caught in a head gate when they were handled through the chute to be either weighed or vaccinated. After blood for lactate measurement and weight were obtained, animals were allowed to leave the chute and the exit velocity was measured with a Farmtek timer system. The initial "infrared" gate was placed

1.824 meters in front of the chute and the final gate was 1.824 meters from the initial gate. Exit speed was recorded and meters per second were calculated for statistical analysis (4).

All steers received the same feed, exposed to the same environment, management and handling throughout their lifespan in an attempt to eliminate confounding variables. The temperament was assessed for all of the steers at weaning, two weeks later when vaccine boosters were administered, when steers were processed into the feedlot, and when weighed half way through the finishing phase just prior to ultrasound assessment to determine harvest day (USDA Choice endpoint). Average Daily Gain (ADG) was calculated using an adjusted 205 weaning weight and weight at midpoint through the finishing phase. (weight at midway through feedlot phase – adjusted weaning weight / 140 days).

The animals were harvested on the same day under federal inspection. A subsample of 15 steers with a low average daily gain (ADG) and a subsample of 15 steers with high average daily gain (ADG) from selected sires were chosen and strip loins collected from each carcass from the designated steers. The loin samples were collected 36 hours after slaughter from the carcasses, and transported to Montana State University (4° C), where loins were cut into steaks and the steaks aged for 3, 7, 14, and 21 days. After samples were aged, they were frozen for further analyses. The samples collected were used to evaluate shear force tenderness and myofibrillar fragmentation index.

Steaks for shear force were thawed at 4°C for 24 h, placed under an electric broiler 10 cm from the heat source and cooked to a final internal temperature of 70°C. Five to eight samples (1.27 × 1.27 × 2.54 cm) from each steak were sheared perpendicular to the fiber direction with a TMS 30 Food Texturometer (Food Technology Corp., Rockville, MD) fitted with a Warner-Bratzler shear attachment (11).

Myofibril fragmentation index (MFI) was determined following the procedures reported by Culler et al (12), as modified by Hopkins et al. (13). Two samples per steer were analyzed and the average of the MFI calculated was used for statistical analyses.

To identify the growth, speed and lactate classifications the mean and standard deviation was calculated using 154 animals for all temperament measurements. The means plus one standard deviation were designated “high” growth, “fast” speed or “high” lactate, and the mean minus one standard deviation represented “low growth, slow speed or low lactate. The rest were designated as medium for the classification. The GLM procedure of SAS was used to analyze tenderness data. The classifications described above were used along with days aged as the independent or class variables. Interactions between days of ageing and growth, speed and lactate class were also tested. The LSMEANS procedure of SAS was used to calculate means. Dunnetts test was used to separate means. Pearson correlations were calculated on all data. Data were considered significant when the P-value was less than or equal to 0.05.

III. RESULTS AND DISCUSSION

Growth classification had a significant effect on shear force values but no effect on MFI. No significant interaction was found between postmortem time and the different temperament or growth classifications. The steers with faster growth rate had steaks with significantly lower shear force values (Table 1). Oddy et al. (14), and Purchas et al., (15) reported that rapid growth over the lifetime of an animal often resulted in lower shear force values. This could be explained by more rapid growth rate resulting in differences in structural and cross linking of the collagen matrix, along with altered proteolytic activity which influences the rate of protein accretion.

Blood lactate classification also significantly affected shear values (P = 0.02). The steaks from steers with medium blood lactate levels were significantly more tender than steaks from steers with high blood lactate. And shear force values of steaks from low and high blood lactate classifications were not different while steads from steers with a medium blood lactate concentration were less tender. This could indicate that high levels of lactate in the muscle ante mortem altered the rate of pH decline enough to impact tenderness. Multiple researchers have reported that altered rates of pH decline postmortem can affect the tenderness of steaks (15, 16).

Table 1: Effect of growth, exit speed and blood lactate classification on myofibrillar fragmentation index (MFI) and shear force values (least squares means).

Class	MFI	Shear
Growth Rate[1]		
Fast	41.4	55.7[b]
Slow	37.4	66.0[a]
P-Value	0.16	0.02
Exit Speed[1]		
Fast	39.6	69.3
Medium	42.1	60.5
Low	36.5	55.7
P-Value	0.27	0.07
Blood Lactate[1]		
High	39.5	57.1[b]
Medium	39.7	67.9[a]
Low	39.5	60.5[ab]
P-Value	0.97	0.02

[1]Classifications for growth rate, exit speed and blood lactate are based on ± one standard deviation from the mean. Plus one standard deviation is fast or high and minus one standard deviation is low. The rest of the steers were classified as medium.
[a,b] means within a column with different superscripts are significantly different P<0.05

A strong trend was noted for exit speed classification to affect shear values. On average steaks from animals that left the chute at a fast speed tended to have higher shear force values than steaks from steers leaving the chute at a medium or slow velocity. This agrees with the work of Cafe et al, (5), Voisinet et al, (8), and Behrends et al, (7) which indicated that steaks from cattle with more excitable temperaments as measured by chute score and exit velocity, had higher shear force values.

Temperament scores were significantly correlated (Table 2). Exit speed was correlated to docility measurement (r = 0.57, P =0.001) and lactate concentrations (r = 0.46, P = 0.01). Temperament measurements were not however, strongly related to tenderness. A significant correlation (r = 0.38, P = 0.04) was observed between the average exit speed and the shear force values after 3 days of postmortem ageing. This suggested that steers with a faster exit speed resulted in meat with higher shear force values. This was supported by (9) who found as temperament increased from calm to excitable, Warner-Bratzler shear force values increased, therefore, concluding temperament had a significant effect on tenderness (9).

As expected a significant correlation ($P \leq 0.02$) was seen between shear force values after 7, 14 and 21 days of postmortem ageing (Table 2). This indicated that if there was a high shear value after 7 days of postmortem ageing there was a higher value after 14 and 21 days of ageing. This suggests that even if shear force values become more similar with postmortem ageing, if they are higher after 3 days of ageing, it will be higher after 21 days of ageing, again contributing to variation in samples in the marketplace.

Table 2: Simple correlations coefficients between (P-value) average docility scores, exit velocity and blood lactate of steers and carcass tenderness measurements (n=30).

	DAVE	SPEED	LAVE	SHR3	SHR7	SHR14
DAVE[1]	1					
SPEED[1]	0.57 (0.001)	1				
LAVE[1]	0.59 (0.0006)	0.46 (0.01)	1			
SHR3[1]	0.15 (0.44)	0.38 (0.04)	0.16 (0.39)	1		
SHR7[1]	-0.11 (0.58)	0.10 (0.60)	0.005 (0.97)	0.70 (<0.0001)	1	
SHR14[1]	-0.10 (0.61)	0.14 (0.45)	-0.005 (0.98)	0.31 (0.09)	0.33 (0.07)	1
SHR21[1]	-0.24 (0.20)	-0.04 (0.84)	-0.12 (0.53)	0.39 (0.03)	0.53 (0.002)	0.26 (0.17)

[1]DAVE= average docility score, SPEED= average exit velocity measurement, LAVE= average blood lactate, SHR= shear force value, 3, 14 and 21 indicate days of ageing postmortem of steaks for shear force

IV. CONCLUSIONS

Growth rate and blood lactate classifications indicated that tenderness was impacted by the altered metabolic environment that occur during the growing phase of animals. Combining temperament data with tenderness data reported here indicate that if an animal is temperamental based on exit scores and lactate concentrations before harvest, this, could predispose a postmortem process that results in a less tender product. Utilizing a simple objective measure of temperament like the lactate meter could possibly lead to decreasing variations in temperament within a producers herd and could lead to less variation in the tenderness a consumer will encounter. However, more research is needed to determine what the optimum blood lactate. Improving the ability of producers to evaluate temperament could result in more consistent tenderness of steaks in the marketplace culminating in a greater consumer satisfaction.

ACKNOWLEDGEMENTS

Research was partially funded by the Bair Ranch Foundation and Montana Experiment Station. The cooperation of Chappell Feedlot and American Simmental Association is also appreciated.

REFERENCES

1. Shook, J. N., D.L VanOverbeke, J.A. Scanga, K.E. Belk, J.W. Savell, T.E. Lawrence, J.B. Morgan, D.B. Griffin, D. S. Hale, and G.C. Smith. 2008. The national beef quality audit–2005, phase I: views of producers, packers, and merchandisers on current quality characteristics of the beef industry. The Professional Animal Scientist 24:189-197.
2. Apple, J. K., Dikeman, M. E., Minton J. E., McMurphy, R. M., Fed, M. R., Leitht, D. E., and Unruh, J. A. 1995. Effects of restraint and isolation stress and epidural blockade on endocrine and blood metabolite status, muscle glycogen metabolism, and incidence of dark-cutting longissimus muscle of sheep. Journal of Animal Science 73:2295-2307.
3. Harvey, S. Phillips, J.G., Rees, A., and Hall, T.R. 1984. Stress and adrenal function. Journal of experimental Zoology. 232:633-645.
4. Curley, Jr., K.O., Paschal, J.C., Welsh, Jr., T.H. and Randel, R.D. 2006. Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. Journal of Animal Science 84:3100-3103.
5. Cafe, L. M., Robinson, D. L., Ferguson, D. M., McIntyre, B. L., Geesink, G. H. and Greenwood, P. L. 2011. Cattle temperament: Persistence of assessments and associations with productivity, efficiency, carcass and meat quality traits. Journal of Animal Science 89:1452-1465
6. Voisinet, B. D., Grandin, T., Tatum, J. D., O'Connor, S. F. and Struthers, J. J. 1997a. Feedlot cattle with calm temperaments have higher average daily gains than cattle. Journal of Animal Science 75:892-896.
7. Behrends, S.M., Miller, R.K., Rouquette Jr., F.M., Randel, R.D., Warrington, B.G., Forbes, T.D.A., Welsh, T.H., Leppke, H., Behrends, J.M., Carstens, G.E. and Holloway, J.W. 2009. Relationship of temperament, growth, carcass characteristics and tenderness in beef steers. Meat Science 81:433-438.
8. Voisinet, B.D., Grandin, T., O'Connor, S.F., Tatum, J.D., and Deesing, M.J. 1997b. Bos indicus-cross feedlot cattle with excitable temperaments have tougher meat and a higher incidence of borderline dark cutters. Meat Science 46:367-377.
9. King, D.A., Schuehler Pfeiffer, C.E., Randel, R.D., Welsh Jr., TH., Oliphint, R.A., Baird, B.E., Curley Jr., K.O., Vann, R.C., Hale, D.S. and Savell, J.W. 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Science 74:546-556.
10. Beef Improvement Federation Guidelines. 2010. Guidelines For Uniform Beef Improvement Programs 9th ed. http://www.beefimprovement.org/PDFs/guidelines/2010%20Guidelines%20,%209th%20Edition.pdf
11. Boles, J.A., Boss, D.L., Neary, K.I., Davis, K.C., & Tess, M.W. (2009) Growth implants reduced tenderness of steaks from steers and heifers with different genetic potentials for growth and marbling. Journal of Animal Science 87:269-274.
12. Culler, R.D., Parrish, F.C., Smith, G.C., & Cross, H.R. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. Journal of Food Science 43: 177-1180.
13. Hopkins, D.L., Littlefield, P.J., & Thompson, J.M. 2000. A research note on factors affecting the determination of myofibrillar fragmentation. Meat Science 56:19-22.
14. Oddy, V.H., Harper, G.S., Greenwood, P.L., & McDonagh, M.B. 2001. Nutritional and developmental effects on the intrinsic properties of muscles as they relate to the eating quality of beef. Australian Journal of Experimental Agriculture 41:921-942.
15. Purchas, R.W., Burnham, D.L., & Morris, S.T. 2002. Effects of growth potential and growth path on tenderness of beef longissimus muscle from bulls and steers. Journal of Animal Science 80:3211-3221.
16. O'Halloran, G.R., Troy, D.J., and Buckley, D.J. 1997. The relationship between early post-mortem pH and the tenderization of beef muscles. Meat Science 45:239-251.
17. Yu, L.P., & Lee, Y.B. 1986. Effects of postmortem pH and temperature on bovine muscle structure and meat tenderness. Journal of Food Science 51:774- 780.

EFFECT ON LAMB MEAT OF SUPPLEMENTING WITH CALCIUM SOAP FATTY ACIDS

Tharcilla Isabella Rodrigues Costa Alvarenga[1*], Iraides Ferreira Furusho Garcia[1], Juan Ramon Olalquiaga Perez[1], Eduardo Mendes Ramos[1], Peter Bitencourt Faria[1], Flavio Augusto Pereira Alvarenga[2], Izac Leopoldino Junior[1], Mario Luiz Chizzotti[3] and David L. Hopkins[4]

[1]Federal University of Lavras, Lavras, Minas Gerais, Brazil
[2]Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[3]Federal University of Viçosa, Viçosa, Minas Gerais, Brazil
[4]NSW Department of Primary Industries, Centre for Red Meat and Sheep Development, Cowra, NSW 2794, Australia
*tharcilla.costa@gmail.com

Abstract – The objective of this study was to determine the effects of calcium soap of fatty acids (CSFA) on meat quality traits of lambs when included in a finishing diet. Cooking loss was reduced in CSFA fed lambs, but there was no effect on any other traits. Samples of *longissimus* muscle were evaluated at 0 and 10 days postmortem. Shear force was significantly reduced by ageing, and this was matched by an increase in myofibrillar fragmentation index values with ageing. *L, *b** and pH values increased with ageing. There was no detrimental effect of feeding CSFA on the measured traits.**

Key Words – lamb, ageing, collagen, shear force

I. INTRODUCTION

At the production level there is pressure for productivity increases and using supplements can increase the energy density of feeds so that ruminants can store more energy with less dry matter consumption. One such potential supplement are calcium soaps. These are produced in a granulated-solid form and are easily incorporated into ruminant diets; affordability will depend largely upon duration and amount of supplementation [1]. Protected lipids are present in an inert form in the rumen; and apparently do not interfere with rumen metabolism, but they are efficiently digested in the lower tract [2] and subject to minimal biohydrogenation [3]. Previous research found no effect on carcass characteristics from including calcium soap fatty acid (CSFA) in the diet of lambs [4]. When high levels of calcium soaps of palm oil fatty acids oil were fed to lambs, ether extract digestibility and the feed conversion ratio were improved without affecting carcass yield and chemical composition [5].

Several studies have evaluated the impact of the diet of ruminants supplemented with CSFA on the composition of ewe milk [6-8], but there is a paucity of knowledge about the impact on meat quality traits. Numerous studies have evaluated the effect of ageing on the tenderness of meat post-mortem [9-11], but whether there is any interaction of CSFA and ageing is unknown. Therefore, the objective of this study was to determine the effects of replacing dietary carbohydrate with CSFA on the meat quality of lambs finished in a feedlot, where the meat was subsequently subjected to different ageing times.

II. MATERIALS AND METHODS

Sixty-three Santa Inês and crossbreed lambs (Santa Inês over Black Dorper, White Dorper, Texel, Lacaune and East Friesan ewes) were used in this study. The feeding period started with lambs weighing on average 26.0 ± 1.06 kg at 3-4 months of age. Twenty-nine lambs were fed with diets containing 85% concentrate and 15% roughage, with the control diet based on oat hay, coffee hulls, corn, soybean meal, limestone, mineral supplement and Rumensin®. The remaining lambs 34 lambs were fed the same diet, but with calcium soap fatty acid Megalac® at 5.4% of the ration, maintaining the isoenergetic and isonitrogenous diets (Table 1). The ME Mcal/kg and crude protein levels were 2.89 and 13.96 for the control diet and 3.11 and 14.00 for the CSFA diet, respectively. Each lamb was fed individually. The lambs were slaughtered after 72 ± 23.85 days on feed and weighed on average 44.0 ± 1.14 kg. The carcasses were kept at room temperature for 6 h and chilled for 18 h at 2-4^{o}C. At 24h postmortem (pm) the carcasses were split down the midline and from the right side of

each carcass the *Longissimus lumborum* (LL) and subcutaneous fat were collected. The muscle was cut into 2.54-cm thick slices and vacuum-packaged. Day 1 pm samples were stored immediately a -20°C for subsequent analysis. Other samples were vaccum packed and stored at 2°C for 10 d pm, then frozen at -20°C for subsequent analysis. The slices were thawed for 18 h at 4°C and the slice used for shear force measurement was weighed individually before and after broiling on a grill for determination of cooking loss. The slice was cooked on a grill to an internal temperature of 71°C, monitored using copper constatan thermocouples. After cooking, slices were cooled at room temperature and four or five 1cm^2 cross-sectional round cores were taken at approximately the same location from each cooked slice and running parallel to the longitudinal axis of the muscle fibers. The cores were sheared on a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., London, UK) with a Warner-Bratzler V-shaped cutting blade that sheared down through the sample and the shear force was recorded as Newtons (N).

Table 1 Ingredients of the experimental diets

Ingredient	Control (%)	CSFA (%)
Oat hay	12.3	12.3
Coffee hulls	2.5	2.5
Corn	69.8	64.8
Soybean meal	12.7	13.7
Megalac®*	-	5.4
Rumensin®	0.02	0.02
Limestone	1.7	0.3
Mineral supplementation	0.9	0.9

*Calcium soap fatty acid (CSFA).

The pH of the sample LL was measured using a Jenco 6009 meter with temperature compensation and an Ionode IJ42 spear electrode, with the electrode inserted into the muscle at 0 and 10 days pm. The electrode was calibrated in buffers at pH 4.0 and 7.0. One steak from LL was allowed to bloom for 30 min at room temperature before color measurement at 0 and 10 days pm. Color measurements were collected using a Minolta CM-700 (Konica Minolta, Japan). A total of 5 readings were taken on each steak and averaged. CIE lightness (L^*), redness (a^*) and yellowness (b^*) were recorded.

Myofibrillar fragmentation index (MFI) was determined as described previously [12]. Sarcomere length was determined on cooked cores samples prior to measurement for shear force as described previously [10]. From each core, sarcomere length of eighteen fiber samples was determined by helium neon laser diffraction (model 05-LHR-073, Melles Griot, Carlsbad, CA) as described previously [13]. Collagen content and heat solubility were determined by Hill's method [14] with slight modification, such as, after cooling to room temperature the extract was centrifuged for 10 min at 3,000 x *g*, the hydrolysis was performed in an oven for 18 h at 105°C and the dilution was 1:10 for supernatant and 1:25 for residual. The amount of hydroxyproline was determined using a procedure described previously [15]. To determine the collagen content, hydroxyproline amount was multiplied by 7.52 for the supernatant and 7.25 for the residual [16]. The supernatant indicated soluble collagen (mg/g muscle) and the residual as the insoluble collagen (mg collagen/g meat). Water holding capacity was determined according to methodology described previously [17] with slight modifications. Briefly, samples of 200-400 mg were placed between filter papers and a 5 kg-weight exerted pressure for 5 minutes. The pressed meat area (MA) and the fluid area (FA) were estimated and the WHC expressed in terms of the ratio of meat to total area, i.e., WHC = MA/(MA + FA).

Statistical analysis: REML Linear mixed models were generated through Genstat 16th Edition [18] using the factors diet (treatment), ageing and the diet x ageing interaction as fixed effects and genotype and animal identification as random terms. pH was tested as a covariate for colour traits, cooking loss and shear force.

III. RESULTS AND DISCUSSION

In our study, there was no significant interaction ($P > 0.05$) between both fixed effects (diet and ageing) for any traits. In Table 2, summary results for pH, b^*, L^*, shear force, cooking loss and MFI are given.

Table 2 Predicted mean and standard error for pH, yellowness (*b**), lightness (*L**), shear force (SF), cooking loss (CL) and myofibrillar fragmentation index (MFI)

Trait	Control		CSFA		Significance (*P* value)	
	0	10	0	10	Diet	Ageing
pH	5.66 ± 0.02	5.67 ± 0.02	5.65 ± 0.01	5.70 ± 0.01	0.544	0.048
*b**	8.9 ± 0.34	9.8 ± 0.34	8.9 ± 0.32	9.7 ± 0.32	0.920	0.008
*L**	48.8 ± 0.71	50.1 ± 0.71	48.2 ± 0.68	49.5 ± 0.68	0.285	0.020
SF (N)	46.8 ± 3.11	33.4 ± 3.11	44.6 ± 2.96	31.1 ± 2.96	0.380	<0.001
CL (%)	25.1 ± 0.61	24.7 ± 0.61	22.8 ± 0.55	23.4 ± 0.55	0.005	0.807
MFI (%)	34.8 ± 2.18	50.0 ± 2.18	35.2 ± 2.07	53.0 ± 2.07	0.285	<0.001

In general, there were no differences due to diet, except for cooking loss with lower values in lambs fed CSFA ($P < 0.05$). In general, a more energetic diet produces rapid gains and fatter carcasses [19]. The degree of cooking loss from muscle tends to decrease with increasing marbling scores [20] and this is a plausible explanation for the effect found in this study.

Ageing was the main factor that affected the meat characteristics, such as pH, *b**, *L**, shear force and MFI. The pH ranged from 5.65 to 5.70, indicating that animals were not stressed at the time of slaughter. According to previous research [21], pH values ranging from 5.5 to 5.8 are normal 24 h after slaughter. The pH value was higher 10 days after the slaughter.

These results may have been due to microbial growth in the vacuum packaging, resulting in elevated pH because of amine compounds produced from bacterial activity in meat proteins may increase pH [22].

Meat colour was influenced by ageing, with the meat being lighter (*L**) and more yellow (*b**) 10 days post mortem (Table 2). Others have shown a positive correlation between ageing time for *L**and *b** [23] and similar to our study they didn't find a significant change in *a** values until after 12 days of ageing attributing this to the short time to bloom (only 20 min), which may not be enough time for maximum red colour development, which is similar to the 30 min bloom time applied in our study.

At 10 days post mortem, shear force measurements were lower than the unaged meat as expected. It is well established that myofibrillar degradation increases during post-mortem meat storage, which can be seen in our study with an increase of 32% in MFI values and a 30% reduction in shear force with ageing for 10 days. The calpains have established effects on muscle during post-mortem storage and play an important physiological role in intracellular protein degradation [11]. There was however, no effect of diet on these traits, although it has been found in previous work that a 22% improvement in tenderness was achieved based on sensory tests when CSFA was added to the diet [24]. Ageing had no impact on collagen solubility.

No differences in meat colour and collagen concentration or solubility due to diet were found (Tables 2 & 3) and this may have been due the similar slaughter weight for all animals (44.0 ± 1.14 kg).

Table 3 Predicted means and standard error for sarcomere length (SL), water holding capacity (WHC), redness (a*) and collagen soluble (Sol) and insoluble (Ins) concentration

Trait	Control	CSFA	*P* value
SL (µm)	1.40 ± 0.02	1.44 ± 0.02	0.20
WHC (%)	0.19 ± 0.03	0.19 ± 0.03	0.20
*a**	10.5 ± 0.33	10.5 ± 0.30	0.98
Sol (mg/g muscle)	0.60 ± 0.03	0.61 ± 0.03	0.56
Ins (mg/g muscle)	1.62 ± 0.05	1.60 ± 0.05	0.76

In Table 3, summary results for sarcomere length and water holding capacity are given. There were no significant effects on these traits due to diet or ageing ($P > 0.05$). The sarcomere length values ranged from 1.40 to 1.44 µm, and this result agrees with results previously presented [10].

IV. CONCLUSION

There was no detrimental effects of feeding CSFA in the diet of lambs, and there was a small beneficial reduction in cooking loss.

Ageing for 10 days produced meat of an acceptable tenderness level, without any adverse effects in the traits measured in this study.

ACKNOWLEDGEMENTS

This work has been financially supported by the Capes, CNPq and Fapemig, as is the post-graduate scholarship for the first author (No. Process: Capes 0360/13-9, CNPq 140508/2011-2) and this is gratefully acknowledged. The authors also acknowledge the contribution of Barbara A. Alves, Rafael A. Marcelino and Fabio L. Cruz (UFLA Brazil), who assisted in measurement of the samples.

REFERENCES

1. Seabrook, J.L., Peel, R.K. & Engle, T.E. (2011). The effects of replacing dietary carbohydrate with calcium salts of fatty acids on finishing lamb feedlot performance, blood metabolites, muscle fatty acid composition, and carcass characteristics. Small Ruminant Research 95:97–103.
2. Salinas, J., Ramırez, R.G., Domınguez, M.M., Reyes-Bernal, N., Trinidad-Larraga, N. & Montano, M.F. (2006). Effect of calcium soaps of tallow on growth performance and carcass characteristics of Pelibuey lambs. Small Ruminant Research 66:135–139.
3. Zinn, R.A., Gulati, S.K., Plascencia, A. & Salinas, J. (2000). Influence of ruminal biohydrogenation on the feeding value of fat in finishing diets for feedlot cattle. Jounal of Animal Science 78:1738–1746.
4. Castro, T., Manso, T., Mantecon, A.R., Guirao, J. & Jimeno, V. (2005). Fatty acid composition and carcass characteristics of growing lambs fed diets containing palm oil supplements. Meat Science 69:757–764.
5. Manso, T., Castro, T., Mantecon, A.R. & Jimeno, V. (2006). Effects of palm oil and calcium soaps of palm oil fatty acids in fattening diets on digestibility, performance and chemical body composition of lambs. Animal Feed Science and Technology 127:175–186.
6. Casals, R., Caja, G., Pol, M.V., Such, X., Albanell, E., Gargouri, A. & Casellas, J. (2006). Response of lactating dairy ewes to various levels of dietary calcium soaps of fatty acids. Animal Feed Science and Technology 131:312–332.
7. Gargouri, A., Caja, G., Casals, R. & Mezghani, I. (2006). Lactational evaluation of effects of calcium soap of fatty acids on dairy ewes. Small Ruminant Research 66:1–10.
8. Dobarganes Garcıa, C., Perez Hernandez, M., Cantalapiedra, G., Salas, J.M. & Merino, J.A. (2005). Bypassing the Rumen in Dairy Ewes: The Reticular Groove Reflex vs. Calcium Soap of Olive Fatty Acids. Journal of Dairy Science 88(2):741-747.
9. Martinez-Cerezo, S., Sanudo, C., Panea, B., Medel, I., Delfa, R., Sierra, I., Beltran, J.A., Cepero, R. & Olleta, J.L. (2005). Breed, slaughter weight and ageing time effects on physico-chemical characteristics of lamb meat. Meat Science 69:325–333.
10. Wheeler. T.L. & Koohmaraie, M. (1999). The extent of proteolysis is independent of sarcomere length in lamb longissimus and psoas major. Journal of Animal Science 77:2444-2451.
11. McDonagh, M.B., Fernandez, C. & Oddy, V.H. (1999). Hind-limb protein metabolism and calpain system activity influence post-mortem change in meat quality in lamb. Meat Science 52:9-18.
12. Hopkins, D.L., Littlefield, P.J. & Thompson, J.M. (2000). A research note on factors affecting the determination of myofibrillar fragmentation. Meat Science 56:19-22.
13. Cross, H.R., West, R.L. & Dutson, T.R. (1981). Comparison of methods for measuring sarcomere length in beef semitendinosus muscle. Meat Science 5:261-266.
14. Hill, F. (1966). The solubility of intramuscular collagen in meat animals of various ages. Journal of Food Science 31(2):161-166.
15. Bergman, I. & Loxley, R. (1963). Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Analytical Chemistry 35:1961-1965.
16. Cross, H.R., Carpenter, Z.L. & Smith, G.C. (1973). Effects of intramuscular collagen and elastin on bovine muscle tenderness. Journal of Food Science 38(6):998-1003.
17. Hamm, R. (1960). Biochemistry of meat hydratation. Advances in Food Research 10:335-443.
18. GENSTAT, (2013). GENSTAT for Windows 16th Edition: VSN International, Hemel Hempstead, UK.
19. Ferrell, C.L., Kohlmeier, R.H., Crouse, J.D. & Glimp, H. (1978). Influence of dietary energy, protein and biological type of steer upon rate of gain and carcass characteristics. Journal of Animal Science 46(1):255-270.
20. Li, C., Zhou, G., Xu, X., Zhang, J., Xu, S. & Ji, Y. (2006). Effects of marbling on meat quality characteristics and intramuscular connective tissue of beef longissimus muscle. Asian-Australian. Journal Animal Science 19(12):1799-1808.
21. Pardi, M.C. Santos, I.F., Souza, E.R. & Pardi, H.S. (2001). Ciência, higiene e tecnologia da carne. (2ªed). Editora da UFG, Goiânia, Brasil, 623p.
22. Rhee, K.S., Krahl, L.M, Lucia, L.M. & Acuff, G.R. (1977). Antioxidative /antimicrobial effects and TBARS in aerobically refrigerated beef as related to microbial growth. Journal of Food Science 62(6):1205-1210.
23. Boakye, K. & Mittal, G.S. (1996). Changes in colour of beef m. longissimus dorsi muscle during ageing. Meat Science 42(3):347-354.
24. Partida, J.A., Olleta, J.L., Sanudo, C., Alberti, P. & Campo, M.M. (2007). Fatty acid composition and sensory traits of beef fed palm oil supplements. Meat Science 76:444–454.

ASSESSMENT OF PORCINE MEAT QUALITY AT THE SLAUGHTER LINE USING RAMAN SPECTROSCOPY

Rico Scheier[1], Martin Scheeder[2], Heinar Schmidt[1]*

[1]Research Centre of Food Quality, University of Bayreuth, Kulmbach, Germany

[2] School of Agricultural, Forest and Food Sciences HAFL, Bern University of Applied Sciences, Zollikofen, Switzerland

*Heinar.schmidt@uni-bayreuth.de

Abstract – Fresh intact porcine *semimembranosus* muscles (N=151) were measured between 30 and 60 min post mortem along the slaughter line of a commercial abattoir using a hand held device to determine the ability of Raman spectroscopy to predict meat quality. Technologically important quality traits such as pH_{35}, pH_{24} and drip loss (DL) were measured using classical reference analysis and they were correlated with the Raman spectra using partial least squares regression. Predicting pH_{35}, pH_{24} and DL yielded a coefficient of determination of 0.75, 0.58 and 0.83 and a root mean square error of cross validation of 0.09 pH-units, 0.05 pH-units and 0.6 %, respectively. For the models, Raman signals of energy metabolites such as lactate, phosphate, ATP and phosphocreatine were weighted. This is the first Raman spectroscopic study to measure and predict quality traits in intact muscles along the slaughtering process showing the potential of early postmortem Raman spectra to measure pH_{35} and to predict pH_{24} and DL.

Keywords – pH, drip loss, early postmortem, non-invasive

I. INTRODUCTION

Meat quality measurements are rarely performed in commercial abattoirs. The reason for this is twofold: (1) The established measuring techniques are either too slow, invasive, imprecise, and/or yield the results only days after slaughter and (2) the spectroscopic methods which were evaluated so far lack the accuracy and/or speed for an on-line assessment of meat quality traits along the slaughtering process.

On the other hand, the relationship between early postmortem metabolism and meat quality is well known and extends beyond extreme deviations such as PSE (pale soft, exudative) or DFD (dark, firm, dry) meat. For example, pH_{45} is correlated with the water holding capacity [1] and the early postmortem metabolism in porcine muscles was shown to influence pH_u, color, drip loss (DL) and shear force [2-6].

In this field, Raman spectroscopy has already been applied to measure the pH in porcine *semimembranosus* (SM) muscles by using only phosphate signals [7]. In addition, the most important spectral changes in early postmortem Raman spectra were assigned to energy metabolites such as lactate, glycogen, ATP and others [8]. Hence, the Raman spectra provide a metabolic fingerprint with the potential to predict the biochemical status. In a previous study, Scheier, *et al.* [9] have used a handheld device to measure and predict quality traits from Raman spectra which were measured between 1 and 2 h *post mortem*. The partial least squares regression (PLSR) models in this study were mostly based on Raman signals of energy metabolites.

However, for an application in abattoirs, the quality assessment has to yield results before the carcasses are entering the chiller. Therefore, in this study, a first attempt is made to apply a mobile Raman device to measure and predict important quality traits at the slaughter line of a commercial abattoir.

II. MATERIALS AND METHODS

Raman and reference measurements were performed as part of a larger study with 151 porcine *semimembranosus* muscles which represent a random sample of pigs slaughtered in Switzerland (48 slaughter batches of different origin).

Early postmortem Raman and pH measurements were performed along the slaughter line 25-60 min p.m. during the normal operation of the abattoir on four days. To this end, 3-5 carcasses at a time were moved from the main slaughter line to the veterinarian line. Then, 3-6 pH measurements were conducted 25-40 min p.m. using a

puncture electrode. Subsequently, seven Raman spectra of meat were obtained from the freshly cut surface of the SM muscle. For the Raman measurements, the mobile system as described in [9] was used. The integration time per spectrum was set to 2.5 s and six spectra were accumulated at each spot. For further analysis, all Raman spectra of each muscle were averaged.

After 24 h, the SM muscles were excised. During the deboning process, 15 samples were lost reducing the number of samples for subsequent analyses to 136. In the laboratory, two replicate pH measurements (pH_{24}) were conducted with a puncture electrode. Then, a 2.5 cm slice of the muscle was weighed, suspended in a box and stored for 48 h. After 3 d, the slices were reweighed and the difference between initial and ultimate weight was expressed as percentage drip loss.

Prediction models for the quality traits were calculated using partial least squares regression, PLS toolbox and MATLAB software.

III. RESULTS AND DISCUSSION

An overview of the results of the reference measurements is given in Table 1. The pH measurements in the abattoir were performed on average ten minutes earlier than the usual pH_{45} measurement, which partly explains the high mean pH of 6.58. The variation in the pH_{35} data as indicated by the standard deviation (SD) of 0.14 pH-units is rather small compared to the within-sample SD of 0.08 (Ref. Error in Table 1) which leads to a low ratio of SD/Ref. error of 1.8 for the pH_{35}.

The pH_{24} values scattered around a mean value of 5.42 with a standard deviation of 0.06. In contrast to the early postmortem pH, the reference error is only 0.02 pH-units, hence the ratio SD/Ref. error of 3.0 indicates a sufficient variance in the pH_{24} data set.

The average drip loss in this data set was 2.8 %. With a maximum value of 5.1 %, the data set contained only one sample with a drip loss above 5 %. Correspondingly, the standard deviation was low. As the drip loss measurement was conducted only once per sample, the reference error of 0.3-1.3 % was estimated from the literature [10]. Due to the margin of 1 %, the ratio SD/Ref. error ranges from 0.7 to 3.0.

Table 1. Overview of measured pH_{35}, pH_{24} and drip loss data and figures of merit for the PLSR models

	pH_{35}	pH_{24}	Drip Loss / %
Mean	6.58	5.42	2.8
SD	0.14	0.06	0.9
Min	6.09	5.30	0.9
Max	6.94	5.65	5.1
Samples	151	136	136
Ref. error	0.08	0.02	0.3-1.3*
R^2	0.75	0.58	0.83
RMSEC	0.07	0.04	0.4
R^2_{cv}	0.55	0.31	0.52
RMSECV	0.09	0.05	0.6

* from [10]

In summary, this data set comprises meat samples of high meat quality as indicated by high pH_{35}, normal pH_{24} and low DL. No deviating samples such as PSE or DFD were found in this random sample.

The Raman spectra were correlated with the pH and DL values using PLSR. The figures of merit of the correlations with the corresponding parameters are presented in Table 1.

The PLSR correlation of the Raman spectra and the pH_{35} values yielded good coefficients of determination with R^2=0.75 and R^2_{cv}=0.55. In Fig. 1, the pH_{35} values calculated from the Raman spectra are plotted against the measured pH_{35} values. Excellent RMSEC and RMSECV were calculated with 0.07 and 0.09 pH-units which are both in the range of the error of the puncture electrode (0.08 pH-units). In principle, the reference error is limiting the predictive ability of the regression model. In comparison to the earlier study [9], the prediction of the early postmortem pH was improved from RMSEC=0.11 to 0.07 pH-units and from RMSECV=0.17 to 0.09 pH-units. This improvement compared to the first study may be attributed to a number of reasons:

(i) a more than four times higher integration time per sample (105 *versus* 25 s) which is improving the signal-to-noise ratio by a factor two,

(ii) a shorter offset between pH and Raman measurement (5-15 *versus* 15-75 min),

(iii) a 50 % shorter period in which the Raman measurements were conducted (30 *versus* 60 min) and

(iv) a larger number of pH measurements per muscle (up to 6 *versus* 2).

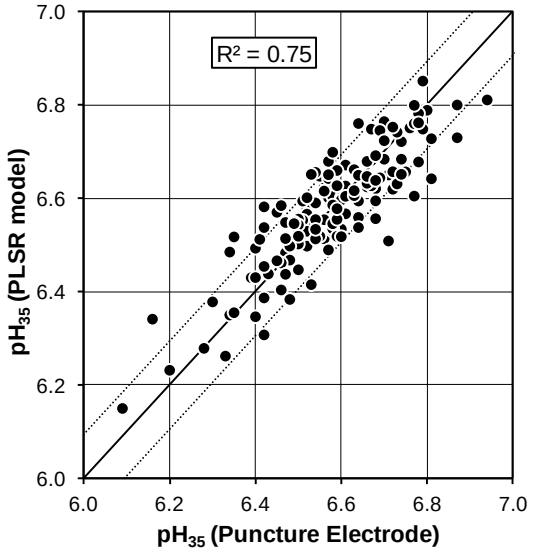

Figure 1. Predicted pH_{35} from Raman data 30-60 min p.m. using PLSR *vs.* pH_{35} measured with pH puncture electrode and RMSECV (dotted line)

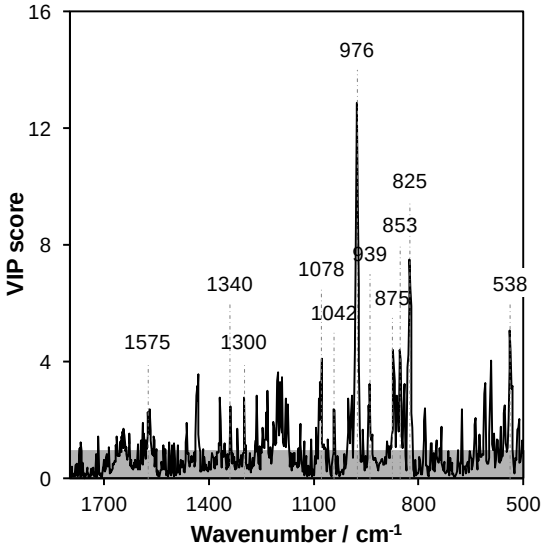

Figure 2. VIP plot of the PLSR model for the prediction of pH_{35} with peak assignments

Variable importance in projection (VIP) plots were used to determine Raman signals being relevant for the prediction of the reference parameter. In case of pH_{35} (see Fig. 2), the VIP plot reveals a strong influence of the phosphate vibration at 976 cm^{-1} and the associated vibration at 1078 cm^{-1} (for peak assignment, see [8]). Peaks at 538, 853 and 1042 cm^{-1} are assigned to lactate, while the peaks at 939 and 1340 cm^{-1} are related to glycogen. The signal at 1042 cm^{-1} in the Raman spectra is partly explained by a small contribution of sugar phosphates, but mostly by creatine, which has a second strong signal at 825 cm^{-1}. The signal at 825 cm^{-1} is weighted as the second strongest signal in the VIP plot indicating the influence of the creatine concentration for the pH_{35} model. This is explained by the high conversion rate of phosphocreatine to creatine in the time frame from 30 to 60 min [11]. Signals of phosphocreatine can be found at 849 and 978 cm^{-1}. The first is superimposed by the strong lactate vibration at 855 cm^{-1}. The partial cancellation of these signals may be the reason for the rather weak VIP score at 853 cm^{-1}. The latter adds intensity to the phosphate signal at 976 cm^{-1}. Besides the small VIP scores at 1300 and 1575 cm^{-1}, no indication for an influence of the ATP concentration can be found in this VIP plot. In conclusion, Raman peaks indicating the current metabolic state are used in combination with the pH-dependent signals of phosphate to calculate the pH_{35} from the spectra.

The previous study [9] has shown that this information can be utilized to predict six quality traits of which, to date, some can only be measured hours or even days after slaughtering. Hence, the spectra were also correlated with pH_{24} and DL.

The PLSR model for the prediction of pH_{24} yields R^2=0.58 and R^2_{cv}=0.31. This is rather poor in comparison to the results of the earlier study [9]. However, this is partly explained by the more than two times smaller standard deviation in the new data set. The PLSR model yields RMSEC=0.04 and RMSECV=0.05 pH-units, which are better compared to the earlier study (RMSEC=0.06, RMSECV=0.09 pH-units). The improved performance can be explained by the higher integration time, the shorter time frame in which the spectra were measured and the smaller reference error of the pH_{24} measurement in this field study.

Again, the VIP plot was used to reveal the most relevant Raman peaks for the pH_{24} prediction (not shown). This model mainly relies on the ATP concentration indicated by a peak at 1124 cm^{-1} and two additional ATP signals Furthermore, signals of lactate, glycogen and inorganic phosphate are weighted by the model.

The PLSR model for the prediction of drip loss yielded R^2=0.83 and R^2_{cv}=0.52. Due to the small variance of the DL data (SD=0.9 %), the coefficients of determination are rather low in this model. However, with RMSEC=0.4 % and RMSECV=0.6 %, its predictive power is

considerably higher than in the earlier study [9].
The VIP plot reveals strong influence of the phosphate concentration and the current pH value with the two phosphate signals at 976 and 1080 cm^{-1}. Besides, signals of lactate, creatine, phosphorylated sugars and ATP are weighted in the PLSR model.

IV. CONCLUSION

In this study, the non-invasive assessment of pH_{35}, pH_{24} and drip loss was shown by using a mobile Raman sensor early at the slaughter line of a commercial abattoir in the time frame from 30 to 60 min after slaughter. Promising prediction models with cross-validated errors in the range of the errors of the reference methods were derived from the Raman spectra. In comparison to a previous study, a significant improvement of the prediction errors (RMSECVs) was achieved amongst other by shorter time slots of the Raman measurements and a shorter delay between pH and Raman measurement. This is underpinning the potential of Raman spectroscopy for an accurate, non-invasive and early quality assessment of porcine muscles in intact carcasses. This could be of interest for an automatable sorting of the carcasses in parallel to the slaughtering process.

ACKNOWLEDGEMENTS

The Research Centre of Food Quality is financed by the European Regional Development Fund (ERDF) for which we are grateful.
We also thank the Micarna SA for the cooperation and the support and the Herman Herzer Foundation for the financial support.

REFERENCES

1. Warriss, P. D. (1982). The relationship between pH45 and drip in pig muscle. Journal of Food Technology 17: 573-578.
2. Henckel, P., Karlsson, A., Jensen, M. T., Oksbjerg, N. & Petersen, J. S. (2002). Metabolic conditions in Porcine longissimus muscle immediately pre-slaughter and its influence on peri- and post mortem energy metabolism. Meat Science 62: 145-155.
3. Monin, G. & Sellier, P. (1985). Pork of low technological quality with a normal rate of muscle pH fall in the immediate post-mortem period – the case of the Hampshire breed. Meat Science 13: 49-63.
4. Bendall, J. R. & Swatland, H. J. (1988). A review of the relationships of pH with physical aspects of pork quality. Meat Science 24: 85-126.
5. Schäfer, A., Rosenvold, K., Purslow, P. P., Andersen, H. J. & Henckel, P. (2002). Physiological and structural events post mortem of importance for drip loss in pork. Meat Science 61: 355-366.
6. Joo, S. T., Kauffmann, R. G., Kim, B. C. & Park, G. B. (1999). The relationship of sarcoplasmic and myofibrillar protein solubility to color and water-holding capacity in porcine longissimus muscle. Meat Science 52: 291-297.
7. Scheier, R. & Schmidt, H. (2013). Measurement of the pH value in pork meat early postmortem by Raman spectroscopy. Applied Physics B 111: 289-297.
8. Scheier, R., Köhler, J. & Schmidt, H. (2013). Identification of the early postmortem metabolic state of porcine M. semimembranosus using Raman spectroscopy. Vibrational Spectroscopy 70: 12-17.
9. Scheier, R., Bauer, A. & Schmidt, H. (2014). Early postmortem prediction of meat quality traits of porcine semimembranosus muscles using a portable Raman system. Food and Bioprocess Technology, In Press, DOI: 10.1007/s11947-013-1240-3.
10. Christensen, L. B. (2003). Drip loss sampling in porcine m. longissimus dorsi. Meat Science 63: 469-477.
11. Kastenschmidt, L. L., Hoekstra, W. G., Briskey, E. J. (1968). Glycolytic Intermediates and Co-Factors in ”Fast-” and ”Slow-Glycolyzing” Muscles of the Pig. Journal of Food Science 33: 151-158.

PROTEOME CHARACTERIZATION OF BUFFALO (*Bubalus bubalis*) AND GOAT (*Capra hircus*) MYOGLOBINS

Naveena B. Maheswarappa[1]*, Usha Rani K.[1], Praveen Kumar Y.[1], Kulkarni, V.V.[1], Kiran, M.[2]

[1]National Research Centre on Meat, Chengicherla, Hyderabad, India
[2]College of Veterinary Sciences, SVVU, Hyderabad, India
*naveenlpt@rediffmail.com

Abstract- This study was conducted to characterize myoglobin (Mb) from water buffalo (*Bubalus bubalis*) and goat (*Capra hircus*) cardiac muscles using two-dimensional gel electrophoresis (2DE) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Purified buffalo and goat Mb samples revealed a molecular mass of 17,043.6 Daltons and 16,899.9 Daltons, respectively. The 2DE analysis of Mb's from buffalo and goat samples revealed 65 (crude Mb) and 6 (pure Mb) differentially expressed spots (P<0.05) between them. Significant variation in 2DE protein spot numbers was observed for crude Mb extract between buffalo and goat samples. Peptide mass fingerprinting (PMF) of Mb protein from 2DE gels confirmed the buffalo and goat Mb's.

I. INTRODUCTION

Water buffalo meat is darker compared to beef and the darkness is attributed to higher Mb content. Darkness of buffalo meat depends on factors other than the oxidation rate of its Mb (1). Goat meat was reported to be darker, more red and had higher sarcoplasmic protein content than sheep meat (2). These observed differences in color attributes between different species indicated possible variation in Mb chemistry.

Recently, using different proteomic tools researchers have demonstrated the effect of muscle source (3) and role of lipid oxidation products (4, 5, 6) on meat color stability. All these studies have reported species-specific effect of Mb on meat color and majority of these studies have used purified myoglobin protein which could differ slightly from the true structure of the native protein in complex biological media. Almost all the studies related to meat color have used liquid chromatography-mass spectrometry (LC-MS) based tools and to our knowledge no studies have been reported on use of two-dimensional gel electrophoresis (2DE) coupled with mass spectrometry to characterize crude and or/purified Mb's especially from buffalo and goat meats. Therefore, present study was undertaken to characterize the Mb using 2DE and mass spectrometry from two important emerging meat animals, buffalo and goat. Study also included the identification of Mb using peptide mass fingerprinting (PMF).

II. MATERIALS AND METHODS

Cardiac muscles from water buffalo and goat were minced, homogenized and subjected to different centrifugation and ammonium sulfate precipitation steps as described by Faustman and Phillips (7). Myoglobin (Mb) pellet was dialysed, filtered using 0.45 µm and 0.2 µm syringe filters and purified through gel filtration column chromatography. SDS-PAGE was performed to check the purity of Mb at each step during the extraction. The purified Mb fraction was subjected to MALDI-TOF MS to determine its intact mass. For 2DE, roughly 200 µl of crude (sarcoplasmic extract) and purified Mb (gel filtered fraction) were loaded on 11 cm immobilized pH gradient strips (pH 3-10), followed by passive rehydration, iso-electric focusing in a Ettan IPGPhor-3 (GE health care, Uppsala, Sweeden) gel apparatus, equilibration and SDS-PAGE (second dimension) with the SE 600 Ruby apparatus as per the procedure standardized at our

laboratory. Gels were stained using Colloidal Coomassie, destained followed by scanning on an Image Scanner III using labscan 6.0 software. Spot detection and quantification were performed with Image Master Platinum7.0 software (GE Healthcare, Uppsala, Sweeden). The myoglobin spot from 2DE gels were also subjected to Trypsin digestion and analyzed on the MALDI TOF/TOF ULTRAFLEX III instrument and further analysis was done with FLEX ANALYSIS SOFTWARE for obtaining the Peptide mass fingerprinting (PMF).

III. RESULTS AND DISCUSSION

Extraction and purification of Mb from buffalo and goat is minimally investigated and to our knowledge only two papers from Dosi et al. (1) and Suman et al. (8) are available in the literature for buffalo and goat Mb, respectively. In the present study, we could able to successfully extract and purify both buffalo and goat Mb's as per the procedure suggested by Faustman and Phillips (7) with 70% ammonium sulfate precipitation and gel filtration chromatography using Sephacryl S-200 HR. The SDS-PAGE of pooled fractions from second peak which is supposed to be Mb consistently revealed the presence of single band at approximately 17 kDa level in both buffalo and goat samples. The MALDI-TOF MS analysis of intact buffalo Mb revealed the mass of 17,043.6 Daltons (Fig. 1A) which is 9.6 Daltons higher than the report of Dosi et al. (2002). The MS analysis of goat Mb revealed a mass of 16,899 Daltons (Fig. 1B).

The 2DE gel analysis (Fig. 2) revealed separation of 508 and 563 spots respectively in buffalo and goat crude sarcoplasmic extracts (crude Mb). The class analysis table by analysis of variance (ANOVA) of buffalo and goat sample gels indicated 65 differential spots which had protein spot expression of 1.5 fold or more between them. For gel-filtered Mb (pure Mb), 19 spots were separated in buffalo relative to 20 spots in goat samples with 6 spots being differentially expressed between them. These findings suggest significant variation in sarcoplasmic proteome between buffalo and goat samples.

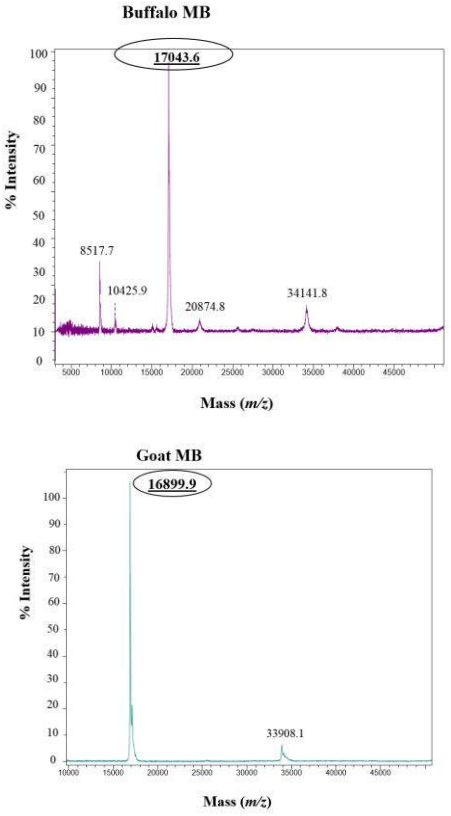

Fig. 1. MALDI-TOF mass spectra of (A) buffalo and (B) goat Mb's

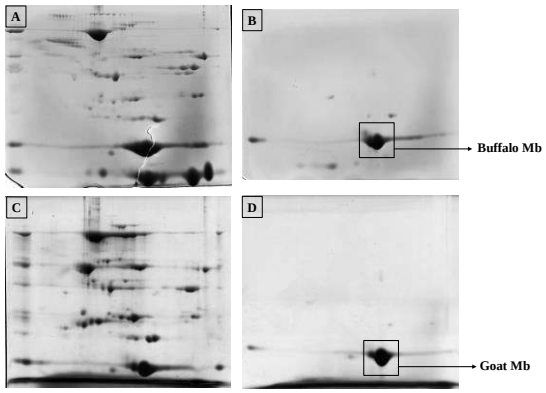

Fig. 2. Two-DE photographs of (A) crude & (B) pure buffalo Mb; (C) crude & (D) pure goat Mb

Purified Mb protein from buffalo and goat samples separated by 2DE gel was identified by peptide mass fingerprinting using MALDI-TOF/TOF mass spectrometry (data not shown). The peptide with a *m/z* value of 748 is most

abundant in buffalo compared to goat samples wherein peptide with 1592 *m/z* is abundant. Peptide masses detection has been matched the buffalo and goat Mb protein in a database which showed the sequence coverage of 39.61% for buffalo and 41.55% for goat. These results confirmed the identity of purified protein spot on 2DE gel as buffalo and goat Mb.

IV. CONCLUSION

Buffalo and goat Mb's were similar in behavior during isolation and purification and exhibited a molecular mass of 17,043.6 Daltons and 16,899 Daltons respectively. The 2DE of crude Mb and purified Mb from buffalo and goat samples revealed significant variation in abundance of proteins between them. The PMF using MALDI-TOF/TOF mass spectrometry of buffalo and goat Mb digested from 2DE gels confirmed the identification of Mb to their respective species. Present study has demonstrated the species-specific variation in 2DE properties of buffalo and goat Mb's

ACKNOWLEDGEMENTS

First author would like thank Department of Science & Technology, Govt. India for financial support (SR/FT/LS-149/2009).

REFERENCES

1. Dosi, R., Di Moro A., Chambery, A., Colonna, G., Costantini, S., Geraci, G. & Parente, A. (2006). Characterization and kinetics studies of water buffalo (*Bubalus bubalis*) myoglobin. Comparative Biochemistry and Physiology, Part B, 145: 230-238.

2. Babiker, S.A., El Khider, I.A. & Shafie, S.A. (1999). Chemical composition and quality attributes of goat meat and lamb. Meat Science, 28: 273-277.

3. Joseph, P., Suman, S.P., Rentfrow, G., Li, S. & Beach, C.M. (2012). Proteomics of muscle-specific beef color stability. Journal of Agricultural and Food Chemistry, 60: 3196-3203.

4. Faustman, C., Liebler, D.C., McClure, T.D. & Sun, Q. (1999). α,β Unsaturated aldehydes accelerate oxymyoglobin oxidation. Journal of Agricultural and Food Chemistry, 47: 3140-3144.

5. Maheswarappa, N.B., Faustman, C., Tatiyaborworntham, N., Yin, S., Ramanathan, R. & Mancini, R.A. (2009). Mass spectrometric characterization and redox instability of turkey and chicken myoglobins as induced by unsaturtaed aldehydes. Journal of Agricultural and Food Chemistry, 57: 8668-8676.

6. Suman, S.P., Faustman, C., Stamer, S.L. & Liebler, D.C. (2007). Proteomics of lipid oxidation induced oxidation of porcine and bovine oxymyoglobins. Proteomics, 7: 628-640.

7. Faustman, C. & Phillips, A.L. (2001). Measurement of discoloration in fresh meat. Ch F3 Unit F3.3. In: Current protocol in food analytical chemistry. New York: Wiley & Sons.

8. Suman, S.P., Joseph, P., Li, S., Steinke, L. & Fontaine, M. (2009). Primary structure of goat myoglobin. Meat Science, 82: 456-460.

IMPROVEMENTS IN FUNCTIONAL PROPERTIES OF DRY CURED MEAT USING FERMENTED SOY PASTE

Abdulatef M. Ahhmed[1,3*], Ceyda Birişik[1], Safa Karaman[1], İsmet Öztürk[1], Mehmet Bilgen[2], Gen Kaneko[3], Hideki Ushio[3], Ryoichi Sakata[4] and Hasan Yetim[1]

[1]Food Engineering Department, Faculty of Engineering, Erciyes University, Kayseri, 38039 Turkey

[2]Department of Biophysics, Faculty of Medicine, Erciyes University, Kayseri, 38039 Turkey

[3] Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan

[4]School of Veterinary Medicine, Azabu University, Sagamihara 252-5201, Japan

*latef.ml@gmail.com

Abstract- Consumers expect less odour and more nutritional value from meat products. Chemen is a wrapping material used in regionally popular dried meat pastirma, but presents a strong odour. To meet the consumer preference, this paper proposes fermented soy paste (Miso) as a potential coating material alternative to chemen. We investigated potential properties of miso to improve the functional, eating quality and nutritional properties of a new product named miso-pastirma (MP). Results suggested that the incorporation of miso as coating material for pastirma has increased both of the fat content and dry matter in MP when compared to commercial pastirma. The increase in the total dry matter indicated that there was an improvement in some of functional properties related to lipids and flavour. MP and M samples were free of the pathogens such as *Salmonella* and *L. monocytogenes.* Results demonstrated that certain proteins were degraded by enzymes derived from meat itself and M that are activated during the processing. All the degradation occurred in MP could improve the flavour, and produce bioactive components that may serve as nutraceuticals. Miso can be an important ingredient for covering pastirma and offers the capacity to improve the functional, nutritional properties and preservation of meat.

I. INTRODUCTION

The Anatolian region has traditional meat products including Kayserian Pastirma which is the most famous and consumed in abundance. Pastirma is a sort of cured meat with an attractive exterior and interior appearances, delicious taste, unique smell and muscle-like shape (1). Pastirma is covered with a paste of grounded spices known as chemen. The mixture of chemen contains 12, 20 % milled fenugreek seeds, crushed garlic, respectively. The potent of garlic and fenugreek in chemen produces strong undesirable odour. Garlic is often called "stinking rose" for its odour as much as for its flavor. After its cells are ruptured, distinctive odours come primarily from sulfur compounds such as allicin that at the end breaks down into diallyl disulfide, which is largely responsible for the garlic's odor. Fenugreek contains an extremely potent aromatic compound called solotone. It can prompt a sweet maple-odour present in sweat and urine. The odour issue can be tackled by dismissing the unpleasant smell: either through using additional chemicals but it may increase the amount of health risk, or through incorporating other flavouring agents into the paste, but at the expense of increasing the cost. So to minimize the level of odour in pastirma, we have been developing new approaches involving natural wrapping materials. In this article, we present our odour masking strategies for improving the flavour and functional properties of the pastirma.

II. MATERIALS AND METHODS

Meat cuts: Meat (*M. latissimus dorsi)* of bulls obtained from a local butcher, Kayseri, Turkey. The pH of the muscle was around 5.6. Samples were kept in refrigerature temperature for 48 hours prior to experimental processes, and the commercial pastirma was also purchased from a local retailer. *Miso*: Miso was purchased from a supermarket in Tokyo area, Japan. It is generally prepared with *koji* (barley grains fermented for 2-3 months by fungi belonging to the *Aspergillus* genus), steamed beans, water, salt, and some starters (*halotolerant* yeasts and lactic acid bacteria).

Protein extraction: Proteins were extracted from samples by adding 28ml of solution (GS-ATP and WSP) to 2g of the samples. Proteins were

also extracted from the fresh samples by adding 5g meat to 20ml D.H_2O. Concentrations of the proteins were determined using the Biuret method.

Proximate analyses and pH values: Ten grams of samples measured using evaporating dish and dried at 105 °C for 4 hours in drying oven (Nüve FN 120, Ankara, Turkey) to determine dry matter of samples. The following equation used to value the dry matter: $DS_2 - D / DS_1 - D \times 100$. (Where D: dish weight, DS_1: dish and samples weight before drying, DS_2: dish and samples weight after drying). Total fat was determined by extracting fat using Soxhelet extraction method. The pH was determined using a pH meter (Mettler Toledo) by embedding probe into minced meat and extracts.

Enumeration of Microorganisms: Twenty-five grams of the sample was mixed with 225mL of sterilized maximum recovery diluents solution and then homogenized using stomacher for 3 min, and then 0.1mL of each dilution was pour plated in specific culture media. Total mesophilic aerobic bacteria were enumerated in the plates of Plate Count Agar (2, 5). Enterobacteriaceae counts were determined using (VRBGA), yeasts and moulds were also enumerated in pour plates of (DRBC) after incubation at 25ºC/5 days and (BPA, Fluka) with egg yolk was used for enumeration of *S. aureus*. *Salmonella* and *L. monocytogenes* (2).

Color measurement: Color measurements of meat cuts and water soluble proteins (WSP) samples were carried out with a Chromometer (Konika Minolta Chromameter CR-5, Japan).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE): SDS–PAGE was used to separate the extracts of proteins according to their size. Electrophoresis was carried out using two different acrylamide gel gradients: 7.5-17.5%. *Fatty acid composition:* The major fatty acids composition was determined with gas chromatography system (Agilent 6890, USA), (3).

Aromatic compound analysis: Aroma analysis performed with slight modifications using GC–MS (Agilent 7890A GC system, Agilent, Avondale, USA) equipped with a mass selective detector and DB-WAX column (60m × 0.250mm) (4).

Texture analysis: The texture analyzer (TA. XT. Plus, Stable Micro-System, Surray, England) was used for measurements of textural properties.

III. RESULTS

Chemen is the wrapping material for pastirma, and it is full of fenugreek and garlic which are delicious ingredients that can greatly enhance a dish, but the smell often comes at the cost of human breath. They have an irritant effect, due to their pungent smell. We tried to get rid of the distinctive smell of pastirma, by incorporating Japanese fruity flavors, the miso taste. The purpose was also to improve the functional and nutritional properties by examining the physicochemical properties of the newly developed product miso-pastirma (MP). Many soy products are being created every year due to research efforts conducted across the world and particularly in Japan.

Proximate: The pH values have not changed much among both pastirma types, slight changes occurred but that was insignificant. The protein content in MP was relatively decreased compared to CP which perhaps refers to the degradation of proteins and peptides into amino acids due to miso action (Table 1). The incorporation of miso as a coating material for pastirma has increased both of the fat content and dry matter in MP. The increase in the total dry matter indicates that there was an improvement in some of functional properties related to lipids and flavour. Results suggest that addition of fermented soy paste may have a positive impact on the dry cured meat products.

Microbial analysis: MP had a higher microbiological count of TMAB. Enterobacteriaceae was under detectable limits in MP and M samples. Yeast was under detectable limits in both MP and M samples. Likewise, mould counts of both samples were under detectable limits. The both samples had insignificant *Enterobacteriaceae* levels. Furthermore, *S. aureus* was detected in the proposed samples, but the contamination level is rather safe. MP and M samples were free of the contamination of the pathogens, *Salmonella* and *L. monocytogenes* (Table 2). The miso covering the pastirma is an important element in terms of both flavour and preservation. Organoleptically, the mixture improves the appearance, colour, texture, taste, and flavour of pastirma, and is also effective against microbial contamination.

Colour analysis: There was a slight difference in *L* values among the tested samples. However, values of *a* and *b* among the three samples did not show any significant difference (Fig. 1). This indicates that miso treatment restored dry meat colour.

SDS-PAGE: Most major small and big water soluble proteins did not retain their native structure as some changes occurred: for instance the WSP, bovine serum albumin 66kDa band disappeared or split to two sub-units in the CP samples (Fig.2). Glutamic dehydrogenase with a molecular weight 55kDa was apparently degraded in MP. Additionally, proteins such as Glyceraldehyde-3-phosphate dehydrogenase 37kDa, Trypsin inhibitor 20kDa and α-lactalbumin 14kDa were degraded in MP samples. All the degradation occurred in MP is a positive rather than negative, as major protein may split into small peptides that could improve the flavour or serve as bioactive components. These results thus demonstrate that certain proteins were degraded by enzymes derived from Miso that are activated during or after the processing. Thus the traditional pastirma-making process results in the degradation of many proteins into peptides, which might then be obtainable to treat some diet-related diseases (1). However, Aprotinin 6.5 kDa existed in all samples CP, MP and M that explains aprotinin is a stable peptide against heat and enzymatic treatment. The trend is going towards foods which not only taste and smells good, but are also beneficial to our health by preventing certain illnesses.

Aromatic analysis: The results indicate that pastirma flavour is formed during protein degradation and derived from some aromatic amino acids because of drying and miso treatment. However, it is likely that additional compounds may also contribute to the overall flavour quality of the MP. In the commercially acquired pastirma, we detected 17 compounds, while miso-pastirma showed 14 compounds. Only one compound was found common, which indicated that pastirma was highly affected by the wrapping materials. Organoleptically, MP also exhibited a fruity and cheesy flavour that is typically derived from miso.

Fatty acid composition: Miso-pastirma showed higher values of miristic, palmitic, stearic, and total saturated fats. Oleic and linoleic acids were higher in CP than MP. However, the commercial pastirma showed higher values of palmitoleic, linolenic, and arachidic acids (Table 3). Content of stearic acid was high in MP which belongs to saturated fatty acids group that is not preferred by nutritionists as affect on human health. Saturated level is high which is related to stearic acid.

IV. CONCLUSIONS

Questionable opinions are always brought up on a large-scale diffusion of meat products supplying potential bioactive components. Consumers recently do not only care about the cost, access and quality of products, simply they have more attention to nutritional values. Incorporation of miso with meat is not a nonsense mixture or confusion of food making, but a unique extension of traditional Japanese miso paste into European meat products to bring healthier and tastier food to the consumer's table. The miso treatment affects the structure of protein and enzyme mechanisms of dry cured meat, and thus potentially increasing the nutritional and sensory values of pastirma. Miso treatment has improved the functional, eating quality, palatability and nutritional properties of dry cured meats, and found to be a good wrapping material alternative to chemen.

REFERENCES

1. Ahhmed, M. A. (2014). Traditional cured meat-making process degrades the proteins of *M. latissimus dorsi* of bovine. Inter. Food Res. J. 21(1): 139-148.

2. AOAC. (1998). Bacteriological analytical manual, FAD administration, 8th Ed. Gaithersburg, MD, USA.

3. Yüksel, F., Karaman S., Kayacier A. (2014). Enrichment of wheat chips with omega-3 fatty acid by flaxseed addition textural and some physicochemical properties, *Food chem*. 145: 910-917.

4. Törnük, F., Karaman S., Öztürk İ. et al., (2013). Quality characterization of artisanal and retail Turkish blossom honeys: determination of physicochemical, microbiological, bioactive properties and aroma profile, *Indus. crops prod* .46:124-131.

5. AOAC (2000) Official Methods of Analysis, (17th Ed.). Association of Official Analytical Chemists, Inc., Arlington, VA.

Table 1. Protein and fat contents and dry matter of commercial pastirma, miso-pastirma and miso.

Sample	Protein content mg/ml		Fat content g/100g		Dry matter g/100g	
	Means	SEM	Means	SEM	Means	SEM
Commercial Pastirma	4.25	-	11.18	2.07	50.25	0.30
Miso-Pastirma	3.66	-	25.25	1.0	80.18	4.12
Miso	1.52	-	5.83	0.65	45.07	0.20

Table 2. Microbial content of miso-pastirma and miso / 0.1mL(diluted samples).

Sample	TMAB	Yeast and Mold	*S. aureus*	Enterobacteriaceae	*Salmonella*	*L. monocytogenes*
Miso-Pastirma	$7x10^3$	$<10^2$	$<10^2$	$<10^2$	0/25 g	0/25 g
Miso	$2x10^3$	$<10^2$	$<10^2$	$<10^2$	0/25 g	0/25 g

TMAB: Total mesophilic aerobic bacteria

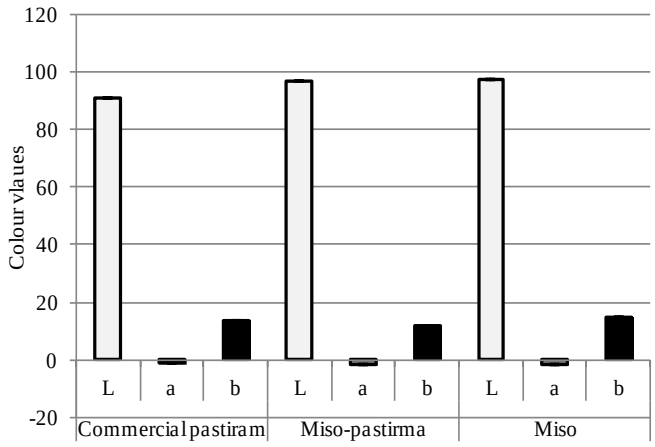

Fig. 1. Colour values of water soluble protein of commercial pastirma, miso-pastirma and miso.

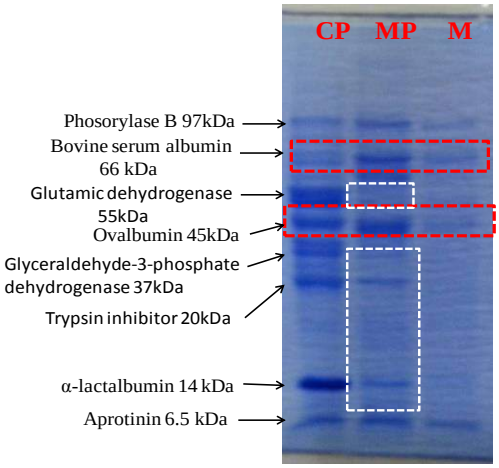

Fig. 2. SDS-PAGE pattern shows the WSP bands in commercial pastirma: CP, miso-pastirma: MP and miso: M. White box shows the absent bands.

Table 3. Fatty acids composition and lipids in commercial pastirma, miso-pastirma and miso.

Fatty acid and lipids	Sample		
	Commercial Pastirma	Miso-pastirma	Miso
Miristic acid (C14:0)	2.95	3.88	-
Palmitic acid (C16:0)	30.63	31.46	12.97
Palmitoleic acid (C16:1)	4.00	3.63	-
Stearic acid (C18:0)	14.32	17.75	4.39
Oleic acid (C18:1)	43.96	41.21	21.96
Linoleic acid (C18:2)	3.71	1.89	52.29
Linolenic acid (C18:3)	-	-	8.39
Arachidic acid (C20:0)	0.42	0.18	-
Total saturated (%)	48.33	53.27	17.36
Total unsaturated (%)	51.67	46.73	82.64

Influence of reduced pressure and physical restraints on pre-mortem tissue swelling which some times results in blood clots. Experiment II

Herbert W. Ockerman and Lopa Basu
The Ohio State University. Columbus, OH, USA.
ockerman.2@osu.edu

Abstract – The influence of reduced pressure on the circumference of foot, ankle and calf was evaluated. Two treatments (sock vs. restraint or support hose) were compared at each airport and at flight elevation. Circumference of the foot, ankle and calf were measured for each evaluation. The data was statistically analyzed for both short and long as well as combined flights. For all flights, and all locations, the lower leg swelled. This swelling was found to be significant for long flights and combined flights for the calf. This would suggest that the restraint is valuable for reducing calf volume under decreased pressure.

Heat sealed plastic not vacuum packaged at airport | Heat sealed plastic swollen at altitude | Bottle capped at altitude | Bottle collapsed at airport

Figure 1. Visual comparisons between objects at the airport and altitude (2 left photographs) and between altitude and airport (2 right photographs)

I. INTRODUCTION

Over 300 million passengers travel each year on long-distance flights (Brenner [4]). High risk passengers for DVT (deep venous thrombosis) include those having previous VTE (venous thromboembolism), known thrombophilia, major surgery within previous 6 weeks, malignancy or family history of VTE, taking estrogen, varicose veins, older age, factor V Leiden mutant ion, joint problems, obesity, inherited or acquired hypercoagulability. This risk increases exponentially with longer duration of travel (> 8 hour flight causes an increase), window or central seats, which suggests that that this problem is a multi causal disorder. A flight as short as 1 hour reduces venous return from legs and leads to local hemoconcentration. A 12 hour flight can lead to swelling and fluid retention which might be expected to confer a thrombosis risk (Watson [10]). In spite of limited research there seems to be only a weak relationship between air travel and VTE but this relationship increases in flyers with increased risk factors and length of flight (Chee [6], Arval [2]). These authors also suggested that future studies are important as the public health concerns are significant. When an aircraft climbs usually to 1500 to 2499 meters the air pressure inside the cabin decreases and air and other gases expand by approximately 30%. These changes do not cause any problems where gas movement can take

place freely, but may cause discomfort or damage when gas is trapped or restricted. Graduated compression stockings {GCS, hosiery; ankle has the greatest pressure, which gradually decreases up to the calf (Agu [1])} are often utilized to prevent flight-related (flight associated, economy class syndrome, coach class thrombosis, flight thrombosis, and travelers thrombosis) thrombosis. The influence of the reduced pressure was not explored until flight-related thrombosis was perceived as a preventable illness. However, the effectiveness of GCS in preventing flight-related thrombosis remains unresolved (Hsieh [7]). This problem is believed to be exacerbated by limited leg space, which can result in compression of veins, especially the popliteal vein (Belcaro [3]) and therefore, reduced calf muscle pump efficiency (Chee [6]). To reduce flight deep vein thrombosis, the most commonly accepted method is the use of external-compression for the prophylaxis of flight-related thrombosis (Byrne [5], Watson [10], Chee [6]). Other intervention recommendations often include standing and moving legs for 5-10 minutes/hour, avoiding baggage between seats, and drinking water regularly (100-150 ml every hour). The results of studies on the effect of GCS ranges form no significant difference from the control to GCS being more effective than no GCS in preventing DVT regardless of the level of risk for DVT (Heish [7]). Several reports suggest that elastic stockings are advised for reducing the rate of DVT (Brenner [4], Scurr [9]). Stockings can alleviate edema which is common during long flights. Edema can be measured by the lower leg circumference. Taking the total reported literature, more studies reported a positive effect than no effect and none reported a negative effect of wearing GCS. No participants in any trials reported complaints or side effects from wearing below the knee GCS and they had good tolerability. A summary by Heish [7] indicated that the application of below-knee GCS significantly decreased the occurrence of DVT but not SVT (other reports in the review indicate it is useful) in long-haul air travelers. Most healthy people when taking long flights experience some swelling of the legs. The airlines recommend exercises and walking to help alleviate this problem; however, it is well known that a closed container will expand when exposed to reduced pressure. After surgery, elastic surgical hose are often used to retard leg swelling. A literature review by Agu [1] examined graduated compression stockings in terms of action, efficiency and complications and recommended that the below-knee rather than thigh-length stocking be used on the basis of equal effectiveness, improved use, patient tolerance and lower cost.

II. MATERIALS AND METHODS

Experiment 1 (Ockerman [8]**)**
Experiment 1 reported in 2013 at the ICoMST in Izmir, Turkey.
Trial 1: A restraint was placed on one leg and a sock on the other. In all locations swelling was reduced significantly or almost significantly.
Trial 2: It could be argued that the body is a closed system and the treatment of one leg might influence the other. In the second trial the same individual using different treatment sequences was evaluated. A restraint was placed on both legs outward bound and a sock was used on the inward bound flight. Even though the restraint meant reduced circumference at all locations for the foot and ankle, the differences were not large enough to be significant. The calf circumference however was significant.
The order of swelling for Experiment 1 was foot, ankle and calf. The elastic restraint retarded swelling at each location and the greatest reduction was in the order calf, ankle and foot. This research suggests that GCS statistically reduces edema on long distance air flights for an individual with medium risk factors. Some swelling occurred in all locations for both restraint and sock treatments and for both short and long flights.

Experiment 2
In Experiment 2 the same individual used a restraint on both legs on the outward bound flight and socks on both legs were worn on the return flight. The flights were also divided into short flights (less than 3 hours) and long flights (more than 3 hours). Also in Experiment 2 since the airports were at different elevations, the difference between the circumference (altitude-airport) at the

foot, ankle and calf were reported instead of the total circumference as was done in Experiment 1.

III. RESULTS AND DISCUSSION

In the current Experiment 2, for both the short and long flights, and with the socks and restraints and at all locations, the swelling differences increased at altitude compared to the airport. For short flights at all locations the decreased swellings were not significant. For long flight however the calf reduction was significant and the foot+ankle+calf reduction was also significant. When long and short flights were combined the same significance was found. This result agrees with the results of Experiment 1. The results of Experiment 2 can be found in Table 1 and are graphically shown in Figure 2.

Both experiments would suggest that on short flights the circumference for all locations would be reduced by the restraint but not enough to be significant (this might change if more data was available). On long flights the calf swelling is reduced significantly for the restraint which is also the case when all flights are combined.

Table 1. Experiment 2 - Results of Statistical Analysis – Difference between altitude and airport measurements in centimeters

	Mean	Std. Dev	P	Mean	Std. Dev	P	Mean	Std. Dev	P
Foot(F)	0.6950	0.7658	0.2756	1.6250	0.8084	0.0742	1.1600	0.8996	0.0676
Ankle(A)	2.5913	0.3566	0.4306	1.9263	0.8418	0.0897	2.2588	0.7127	0.0865
Calf(C)	3.6963	0.6142	0.5717	5.3329	2.5594	*0.0307	4.4600	1.9262	*0.0368
F+A+C	2.3275	1.3910	0.5230	2.8583	2.2444	*0.0242	2.5872	1.8570	*0.0241

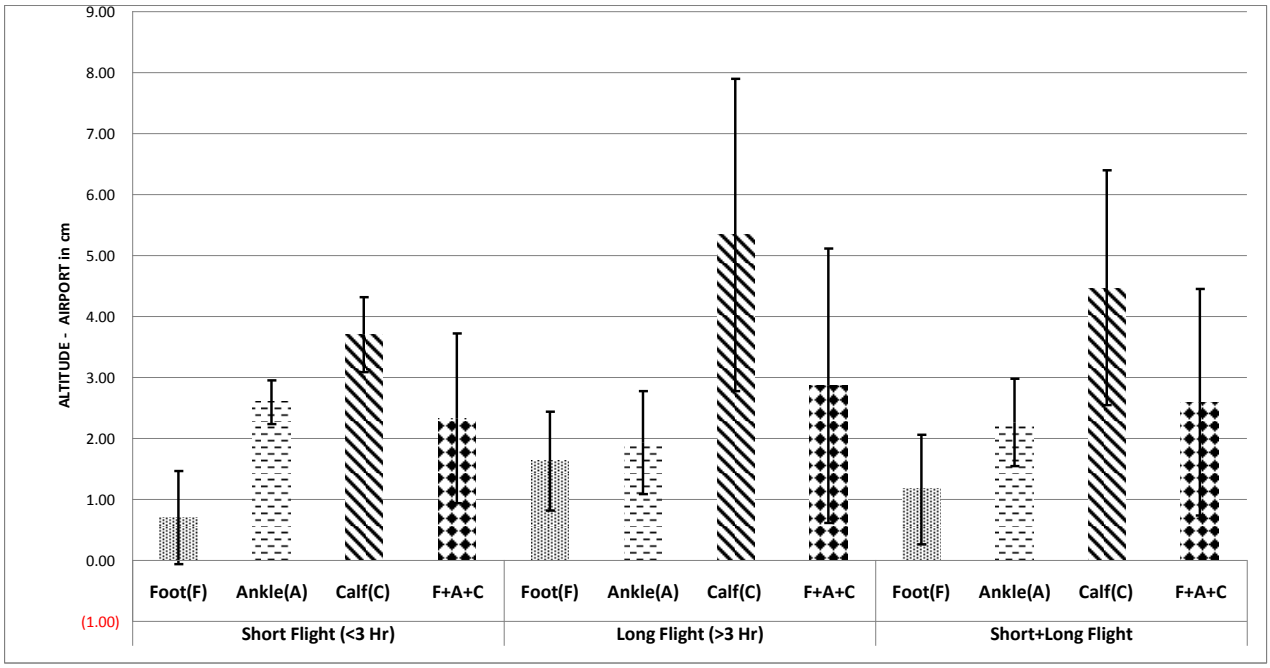

Figure 2. Mean difference in circumference (altitude - airport) for each location and for short and long flights and combined flights

CONCLUSIONS

In all cases and at all locations, restraints reduced swelling. However, this was only significant in the calf area and on long flights. Increase in sample size might result in more locations being significant.

REFERENCES

1. Agu O, Hamilton G, Baker D. Graduated 1999 Compression stockings in the prevention of venous thromboembolism. Brit J Surg;86(8):992-1004

2. Arval K. R. and Al-Khaffat 2006. Venous Thromboembolic Complications Following Air Travel: What's the Quantitative Risk? A literature Review.2005. European Journal Endovasc Surg 31;187-177.

3. Belcaro G., Casarone M.R. Nicolaides A. N., Ricca A., Geroulakos G., Shah S.S. G., Ippolito E., Mayers K.A.Bavera P., Dugall M., Moia M., Di Renzo A., Erricfhi B. A., Brandololini R., Griffin A., Ruffini I., and Aseribi G 2003 Prevention of venous thrombosis with elastic-stockings during long flights: The LONFLIT 5 JAP study. Clinical and Applied Thromostasis 9,197-201.

4. Brenner B. 2006. Intervention to prevent venous thrombosis after air travel, are they necessary? Yes. J. Thromb Haemost 4:2302-5.

5. Byrne B. 2001. Deep vein thrombosis prophylaxis: the effectiveness and implications of using below-knee or thi-length graduated compression stockings. Heart and Lung 30,277-284.

6. Chee Y. L. and Watson H. G. 2005 Air Travel and Thrombosis. British Journal of Haematology 130: 671-680.

7. Hsieh, Hsiu-Fang and Lee, Feng-Ping 2005. Graduated compression stocking as prophylaxis for flight-related venous thrombosis: systematic literature review. J. of Advanced Nursing 51(1)82-98.

8. Ockerman H.W., Basu L.2013, 59th CoMST Izmir Turkey ,I S6B-8 - Influence of Reduced Pressure and Physical Restraints on Pre-mortem Tissue Swelling Which Some Times Results in Blood Clots.

9. Scurr J. H., Machin S. J., Bailey-King S., Mackie I. J., McDanald S., and Coleridge Smith P. D., 2001. Frequency and prevention of symptom less deep-vein thrombosis in long-haul flights: a randomized trial. Lancet 357:1485-9.

10. Watson H. G. 2005. Travel and thrombosis. Blood reviews 19, 235-241.

RELATIONSHIPS BETWEEN FIBER TYPING AND MEAT TENDERNESS IN 15 HEAVY LAMB MUSCLES

Javier Ithurralde[1*], Gianni Bianchi[2], Oscar Feed[2], Fernando Nan[1], Fernando Ballesteros[2], Gustavo Garibotto[2] and Alejandro Bielli[1].

[1] Area of Histology and Embryology, Dept. of Morphology and Development, Veterinary Faculty. Lasplaces 1550, Montevideo, Uruguay.

[2] Research station "Dr. Mario A. Cassinoni" (EEMAC) Agronomy Faculty. Route 3, km 363, Paysandú, Uruguay.

*javiithu@gmail.com

Abstract- The aim of this study was to describe the relationships between fiber typing and sarcomere length, instrumental and sensory meat tenderness in 15 heavy lamb muscles, as well as in selected homogeneous groups of muscles. Samples were taken from muscles *Semitendinosus*, *Longissimus lumborum*, *Longissimus thoracis*, *Semimembranosus*, Cranial *Gluteobiceps*, *Adductor*, *Gluteus medius*, *Triceps brachii caput longum*, *Triceps brachii caput laterale*, *Psoas major*, *Rectus femoris*, *Vastus lateralis*, *Serratus ventralis*, *Infraspinatus* and *Supraspinatus* of five crossbred heavy lambs. Contractile fiber types were revealed histochemically, and muscles were arbitrarily classified according to the percentage volume of slow fibers. Sarcomere length, instrumental (WB-shear force) and sensory tenderness were determined 24 h after slaughter in all muscles. Although significant correlations were detected between fiber typing and meat quality across all (pooled) muscles, grouping muscles according to their fiber type characteristics resulted in finding important intermuscular differences in the way fiber typing and meat quality were associated. Therefore, our results suggest that in heavy lambs, associations between fiber typing and meat quality vary depending on muscle fiber type characteristics.

I. INTRODUCTION

Although it is accepted that muscle fiber diversity can affect meat quality, identification of a superior fiber type composition for meat quality has not been reported and may vary between species (1).

In the ovine species, some studies report associations between fiber typing and meat tenderness (2-3) whereas some do not report such associations (4).

Furthermore, since meat quality is affected by a complex combination of intrinsic and extrinsic factors, such as postmortem pH decline rate, muscle temperature and buffering capacity (5-6), some of which can show intermuscular variations (7), we hypothesize the existence of some degree of heterogeneity (muscle-type dependent) in the way fiber typing and meat quality is associated.

Thus, the aim of this study was to describe the associations between meat tenderness and fiber typing in 15 heavy lamb skeletal muscles as well as in selected contractile groups of muscles.

II. MATERIALS AND METHODS

Five 14-month-old Poll Dorset crossbred heavy-ram lambs (71.9 ± 1.67 Kg) were used. Immediately after slaughter, samples were taken from the mid superficial belly of the *Semitendinosus*, *Longissimus lumborum*, *Longissimus thoracis*, *Semimembranosus*, Cranial *Gluteobiceps*, *Adductor*, *Gluteus medius*, *Triceps brachii caput longum*, *Triceps brachii caput laterale*, *Psoas major*, *Rectus femoris*, *Vastus lateralis*, *Serratus ventralis*, *Infraspinatus* and *Supraspinatus* muscles. Samples were frozen in liquid nitrogen, included in cryostat embedding medium (Cryomatrix, Thermo Shandon Limited, USA), and 24 µm-thick sections were cut in a cryostat. Histochemical fast (type II) and slow (type I) fiber types were revealed using the mATPase stain after alkaline (pH=10.35), and acid (pH=4.35) preincubations (8). The proportions, mean diameters and percentage volumes (%V) of type I and II fibers were determined using an image analysis system (Infinity analyze®, Toronto, Canada). An arbitrary criterion was followed to classify muscles according to their contractile characteristics. Muscles in which the %V occupied by type I fibers was lower than 10% were classified as fast twitch muscles. Muscles in which the %V occupied by type I fibers was between 10 and 20%, were considered intermediate twitch muscles, while muscles in which type I %V was higher than 20% were classified as slow twitch muscles. Sarcomere length was determined histologically in 2.5 % glutaraldehyde fixed samples. Instrumental tenderness was determined in cooked meat through Warner Bratzler (WB) shear force with an Instron series 3342. Sensory tenderness was determined through consumer panel sessions including 180 consumers (110 male and 70 female). A balanced incomplete block design and a ten point discontinued scale were used. Pearson correlation coefficients between fiber typing and meat quality traits were generated across all (pooled) muscles, as well as within selected contractile homogeneous groups of muscles.

III. RESULTS AND DISCUSSION

III.I. Muscle classification

Fast twitch muscles included muscles *Semimembranosus*, *Rectus femoris*, *Semitendinosus* and *Longissimus lumborum*. Muscles classified as intermediate twitch were Cranial *Gluteobiceps*, *Triceps brachii caput longum*, *Vastus lateralis*, *Gluteus medius*, *Longissimus thoracis* and *Adductor*, while slow twitch muscles included muscles *Serratus ventralis*, *Infraspinatus*, *Supraspinatus*, *Psoas major* and *Triceps brachii caput laterale* (Fig. 1).

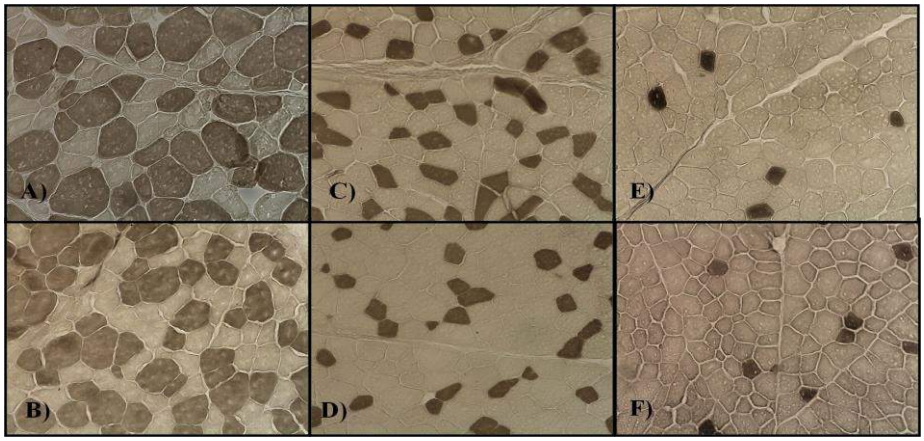

Fig.1. Histological sections of different heavy lamb muscles treated with the myosin ATPase stain after acid pre-incubation. A (*Serratus ventralis)* and B (*Supraspinatus*): slow twitch muscles; C (*Triceps brachii caput longum)* and D (*Adductor*): intermediate twitch muscles; E (*Semitendinosus)* and F (*Longissimus lumborum*): fast twitch muscles. Slow fibers stain dark while, fast fibers stain light beige.

III.II. Correlations between fiber typing and meat quality traits

The correlations between fiber typing and meat tenderness found in all (pooled) muscles (Table 1) are consistent with the higher calpastatin activity of slower muscles (4). Moreover, the detected intermuscular differences regarding correlations between meat tenderness and fiber typing (Table 1), suggest that although fiber typing can be useful to explain intermuscular differences in meat tenderness within a large and heterogeneous group of muscles, these relationships may not necessarily apply to smaller and more homogeneous groups of muscles. This could be one of the reasons why in studies covering a reduced number of muscles, no clear associations are apparent between fiber typing and meat tenderness. In fact, within muscles *Longissimus dorsi*, *Tensor fasciae latae*, *Semitendinosus*, *Trapezius* and *Supraspinatus* of rams, no correlations were found between fiber typing and meat tenderness (4).

Regarding sarcomere length, our results comprising all muscles disagree with the theory which states that slow fibers are associated with shorter sarcomeres (9). Nevertheless, Aalhus and Price (10) reported that among different ovine muscles, fiber typing could not predict sarcomere lengths, since the degree of stretching during hanging would influence this relationship. However, in the present study sarcomere length proved to be a variable with markedly different behavior depending on the muscle group. This could help to explain why in studies covering a heterogeneous group of muscles no negative association can be found between slow fibers and shorter sarcomeres (11).

Table 1: Pearson correlation coefficients between fiber typing and meat quality traits in: all (Pooled), slow twitch (SlTw), intermediate twitch (InTw) and fast twitch (FsTw) muscles.

	WB shear force				Sensory tenderness				Sarcomere length			
	Pooled	SlTw	InTw	FsTw	Pooled	SlTw	InTw	FsTw	Pooled	SlTw	InTw	FsTw
% Type I					-0.29 **	-0.62 ***						
Type I mean diameter	0.38 **	0.71 **			-0.36 **	-0.48 *	-0.45 **				0.52 **	-0.77 ***
% V type I					-0.31 **	-0.53 **	-0.59 ***					-0.77 ***
Type II mean diameter									-0.36**			

*P<0.1; **P<0.05; ***P<0.01.

IV. CONCLUSION

The present study contributes to a better understanding of the influence of fiber typing on intermuscular meat quality diversity, suggesting that although fiber diversity may explain intermuscular differences in meat tenderness, these associations can also vary among different contractile groups of muscles.

REFERENCES

1. Lefaucheur, L., & Gerrard, D. (2000). Muscle fiber plasticity in farm mammals. Journal of Animal Science, 77, 1-19.
2. Valin, C., Touraille, C., Vigneron, P. & Ashmore, CR. (1982). Prediction of lamb meat quality traits based on muscle biopsy fibre typing. Meat Science, 6, 257-263.
3. Solomon, MB. & Lynch, GP (1988). Biochemical, histochemical and palatability characteristics of young ram lambs as affected by diet and electrical stimulation. Journal of Animal Science, 66, 1955-1962.
4. Sazili, AQ., Parr, T., Sensky, PL., Jones, SW., Bardsley, RG. & Buttery, PJ. (2005). The relationship between slow and fast myosin heavy chain content, calpastatin and meat tenderness in different ovine skeletal muscles. Meat Science, 69, 17–25.
5. Dransfield, E. (1993). Modelling post-mortem tenderisation – IV: role of calpains and calpastatin in conditioning. Meat Science, 34, 217–234.
6. Huff-Lonergan E. & Lonergan, SM. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science, 71, 194–204.
7. Purchas, RW., Rutherfurd, SM., Pearce, PD., Vather, R. & Wilkinson, BHP. (2004). Concentrations in beef and lamb of taurine, carnosine, coenzyme Q(10), and creatine. Meat Science, 66(3), 629–637.
8. Dubowitz, V. & Brooke, MH. (1973). Muscle Biopsy: A Modern Approach. W. B. Saunders, Philadelphia, PA.
9. Hertzman, C., Olsson, U. & Tornberg, E. (1993). The influence of high-temperature, type of muscle and electrical-stimulation on the course of rigor, aging and tenderness of beef muscles. Meat Science, 35, 119−141.
10. Aalhus, JL. & Price, MA. (1991). Endurance-Exercised Growing Sheep: I. Post-mortem and Histological Changes in Skeletal Muscles. Meat Science, 29, 43-56.
11. Hwang, YH., Kim, GD., Jeong, JY., Hur, SJ. & Joo, ST. (2010). The relationship between muscle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korean native cattle) steers. Meat Science, 86, 456–461.

"META-ANALYSIS OF THE EFFECT OF DIETARY SELENIUM SUPPLEMENTATION ON GLUTATHIONE PEROXIDASE ACTIVITY IN POULTRY"

M. Laura Alberro[1]*; M. Cristina Cabrera[1,2] & Ali Saadoun[2]

[1]Laboratorio de Nutrición & Calidad de Alimentos, Facultad de Agronomía, UDELAR, Montevideo, Uruguay.
[2]Sección Fisiología & Nutrición, Facultad de Ciencias, UDELAR, Montevideo, Uruguay.
*mlalberro@gmail.com

Abstract - The term Glutathione Peroxidase (GPx) defines a family of enzymes in the antioxidant system of aerobic organisms. Among them we find the seleno-enzymes, which contain Selenium (Se) in their active site as a fundamental factor in the efficiency of the enzyme's activity. Because of its pivotal role, supplementing animal diets with Se has become a common practice in farming. In the present work a meta-analysis (MA) of means was performed on the results of a literature search of electronic databases, complemented with a one way analysis of variance (ANOVA). The objective was to evaluate and compare the tendency of the effects produced by supplementation with different doses and sources of Se on the activity of Se-dependant GPx in the blood of poultry, which were initially fed either with a basal Se or Se deficient diet. The results showed a significant increase in enzymatic activity only when the initial diet was deficient in Se. Only one group showed a significant difference between organic and inorganic Se. From these observations it can be concluded that when the initial diet has a normal Se content, supplementation with Se would not produce a significant effect on GPx activity.

I. INTRODUCTION

Glutathione Peroxidase (GPx) is a family of enzymes in the antioxidant system of aerobic organisms. It catalyzes the reaction in which reduced Glutathione reacts with peroxides to transform them to water and alcohol. GPx has the capacity to protect the integrity of unsaturated bonds of membrane phospholipids by extinguishing free radical attacks capable of initiating and propagating lipid oxidation (1).

The main form of GPx is Selenium (Se) dependant and uses it as a cofactor. The importance of Se in animal nutrition lies in the fact that several levels of antioxidant defence rest on the Se-dependant GPx activity, which depends on an adequate Se status in the cell.

Lipid oxidation is accelerated when there is a deficiency in Se (2). There are different mechanisms through which the antioxidant system can be altered or regulated. The main one is the synthesis of antioxidant enzymes (e.g. SOD and GPx), as the animal responds to stress conditions. However, this response will only be effective if the cofactors needed for the enzymatic action are available. Therefore, the nutritional intake of Se is a crucial factor in the regulation of GPx activity and the efficiency of the antioxidant system (3).

Lipid oxidation is one of the main factors limiting the quality and acceptability of meat and meat products. It leads to discolouration, drip losses, off-odour and off-flavour development, and the production of potentially toxic compounds (4). There are several critical phases of lipid oxidation: the balance between production of reactive oxygen species and the antioxidant defence system in the living animal; oxidative damage in the immediate post-slaughter period and oxidation during handling, processing, storage and cooking.

There are indicators that show that dietary supplementation with selenium, particularly organic selenium, improves quality and shelf life of poultry meat. Studies on chicken and turkey have shown that organic selenium is deposited more effectively in muscles than inorganic selenium. The increased tissue concentrations of selenium not only decreases oxidative stress, including protecting unsaturated fatty acids from peroxidation damage, but can also reduce drip loss from meat and the incidence of pale soft, exudative meat. It also improves the shelf life during refrigeration. (5)

Meat colour and quality analysis in a study by Yang (6) showed that birds fed with diets

containing 0.3 ppm of organic Se had a decrease in cooking loss and myoglobin oxidation, and an increase in the degree of red colour in the meat. These findings suggest that Se-enriched yeast improved the antioxidative status of broilers by increasing the activity of antioxidant enzymes, hence dietary Se supplementation, especially with organic Se, may have a beneficial effect on the oxidative stability and extended shelf life of fresh meat.

In the present study the effect of Se supplementation on GPx activity was analysed for different species and tissues in the following diverse situations: initial diet deficient in Se; initial basal diet with normal Se content and diets supplemented with organic or inorganic sources of Se.

The objective of the analysis was to study the tendency of the effect of dietary supplementation with Se on GPx activity in animals in different situations, performing the relevant comparisons between groups. Only the results where GPx activity was measured in poultry blood are shown.

II. MATERIALS AND METHODS

A literature electronic search was performed to identify the relevant studies. The EBSCO meta-search service was used to search in the following databases: Academic Search Complete, CAB Abstracts, Directory of Open Access Journals, MEDLINE, Ovid Journals, Science Direct and Scopus, using the key words: GPX (abstract) AND Selenium (abstract) AND muscle (all text) without restrictions of date, language or type of document. In addition, a search for "grey literature" was performed through the search engine Google to minimize publication bias, which can happen when searching exclusively for literature published in refereed journals.

Then the literature retrieved was filtered according to inclusion and exclusion criteria, which were previously set to ensure the articles were methodologically homogeneous. Subsequently the quality of the studies used in the meta-analysis was evaluated using as a guideline the table described by Berman (7). The parameters described there were adapted according to the conditions of the present study.

The programme NCSS 2007 was used for statistical processing of the data. A meta-analysis of means was performed, with $\alpha = 0.05$ and a One Way ANOVA for each group to complement the meta-analysis, studying GPx activity vs. Se dose and GPx activity vs. type of Se (organic or inorganic). For the latter the Tukey-Kramer Multiple Comparison Test was used.

Homogeneity was evaluated using the Cochran's Q Test and the Radial Plot (Galbraith Plot).

Publication bias was assessed with a Funnel Plot, using the graph Standard Error vs. mean of Se supplemented GPx Activity.

III. RESULTS AND DISCUSSION

The obtained measurements were grouped according to their species, tissue, initial diet and dose of Se supplementation. This paper shows the results of two of the four groups analysed, which measured GPx activity in blood of poultry (laying hens, broiler chickens and turkeys). The groups are as follows:

Group 1: Poultry, without stress, initial basal diet in Se supplemented with low-medium dose of Se (between 0.01 and 0.46 ppm).

Group 2: Poultry, without stress, initial diet deficient in Se supplemented with low-medium dose of Se.

Even though data was obtained for several different tissues in each group, it was only possible to perform the MA for those tissues which had a minimum of six measures.

The heterogeneity test was performed on the different groups and the result was significant for each of them, showing that the data was heterogeneous in all cases. This was confirmed by the Radial Plots; therefore the Model of Random Effects was used to perform each MA.

Figure 1 shows the Forest Plot for group 1. This indicates that there are non-significant results (where the confidence interval line intersects the vertical line 0.0), but the average measure is significant. The size of the symbol is correlated with the weight each study has in the MA.

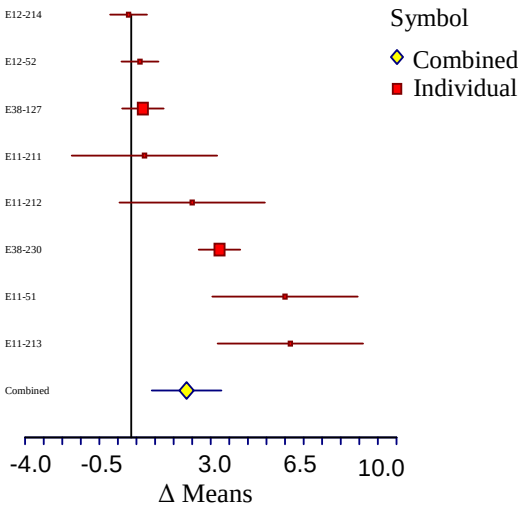

Fig. 1. Forest Plot Group 1

Comparing the MA results for Group 1 and Group 2 verifies that for Group 1 the GPx activity does not increase by the same amount as Group 2, which correlates with the study design. Though in both cases the Se supplementation dose was low-medium, Group 1 started with a basal diet (normal Se intake) while Group 2 started with a diet deficient in Se. The MA forest plot for Group 2 is shown in figure 2.

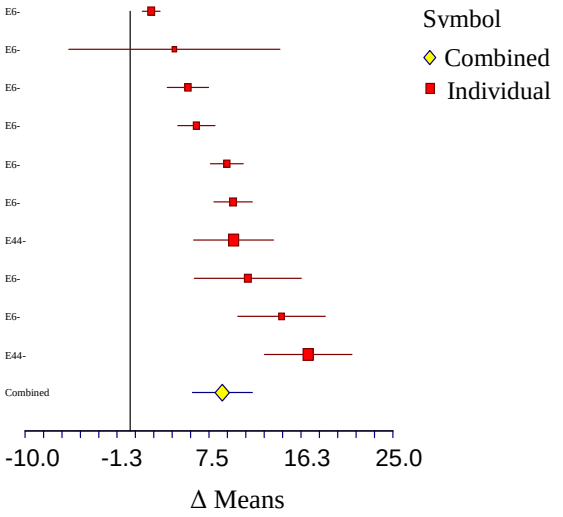

Fig. 2. Forest Plot Group 2

To complement the meta-analysis, a One Way ANOVA (OWAV) was performed on each group to study the effect on GPx activity of the dose of Se supplementation and type of Se (organic or inorganic).

The increase in GPx activity based on Se dose for Group 1 was not significant (probability level > α), which correlates with the study design; animals in Group 1 started from a diet with a normal Se content. From the Tukey-Kramer Multiple Comparison Test we observe that there are no significant differences between the control group and group 1, which coincides with the results of the MA. There is no increase in the activity of GPx based on Se dose, neither for inorganic Se nor for organic Se.

For Group 2, the increase in GPx activity based on Se dose was significant (probability level < α), which corresponds with the study design, because this group was fed initially with a diet which was deficient in Se. The results for the Tukey-Kramer test show that the group with a Se deficient diet differ significantly from the supplemented group. It also shows a significant difference between both extremes in the range of the evaluated supplementation; with higher supplementation having a greater effect.

The results also show that there is a significant difference between the control group (deficient in Se) and the supplemented groups (specifically with inorganic Se), which is in accordance with the result of the meta-analysis performed for this group. There is no significant difference between the group supplemented with organic Se and the one supplemented with inorganic Se.

IV. CONCLUSIONS

According to the results obtained, we observe that when we start from a basal diet with a normal Se content, there is no effect produced by supplementation, as we see in Group 1. This may be due to the better oxidative stability caused by sufficient intake of Se, which without stress in the study design, causes a lower need for the synthesis of the antioxidant enzyme (8) (9). However, when we start from a diet deficient in Se, we can see a clear effect caused by Se supplementation in the diet on GPx activity, as is evident in Group 2. In addition, in this group a significant difference was observed between the results of adding different doses of Se, increasing the enzyme's activity to a higher degree when supplementing with a higher dose. Several articles (10) (11) state that if the initial diet is deficient in Se, when the dose is increased, the GPx activity also increases until it reaches a well defined plateau, therefore any further

increase in the dose will not produce a greater effect.

Regarding the different sources of Se, in Group 1 there was no difference observed between the animals fed with a diet supplemented with organic Se and the ones fed with a diet supplemented with inorganic Se. However, in Group 2 the increase in enzymatic activity was only significant when inorganic Se was administered. With respect to this observation, there is much controversy among researchers. Some of them comment that inorganic Se is metabolised more effectively and as a result GPx activity increases to a higher degree than when organic Se is supplied (12) (9). Others obtained results that favour the supplementation of organic Se over inorganic, based on a more effective absorption of Se in the intestine when it is attached to an organic source (1) (13) (6).

There are many known factors that affect the GPx activity apart from dietary Se. These include age, sex and ambient variables that can produce stress. It is interesting to note that some studies in young chickens on growth response to nutritional Se deficiency showed that these had a hereditary component (14). As a result, the consequences of Se nutritional deficiencies in animals could differ among genotypes. Therefore, it is of paramount importance to take into account all factors involved in a particular study to thoroughly understand and evaluate the results.

V. REFERENCES

1. Mikulski D.; Jankowski J., Zdunczyk Z., Wroblewska M., Sartowska K., Majewska T. (2009). The effect of selenium source on performance, carcass traits, oxidative status of the organism, and meat quality of turkeys. Journal of Animal Feed Sciences, 18:518-530.
2. Halliwell B., Gutteridge J. M. C. (1999). Free radical in biology and medicine. 3rd. ed. Oxford University Press.
3. Surai, P. F. (1999). Tissue-specific changes in the activity of antioxidant enzymes during the development of the chicken embryo. Br. Poult. Sci. 40, 397-405.
4. Morrisey P.A., Sheehy J.A., Galvin K., Kerry J.P., Buckley D.J. (1998). Lipid stability in meat and meat products. Meat Sci. 49:S73-S86.
5. Mahima. (2012). Inorganic versus organic Selenium supplementation: A review. Pakistan Journal of Biological Sciences 15 (9): 418-425.
6. Yang Y.R., Meng F.C., Wang P., Jiang Y.B., Yin Q.Q., Chang J., Zuo R.Y., Zheng Q.H., Liu J.X. (2012). Effect of organic and inorganic Selenium supplementation on growth performance, meat quality and antioxidant property of broilers. African Journal of Biotechnology Vol. 11(12), 3031-3036.
7. Berman N.G., Parker R.A. (2002). Meta-analysis: Neither quick nor easy. BMC Medical Research Methodology 2:10.
8. Leeson S., Namkung H., Caston L., Durosoy S., Schlegel P. (2008). Comparison of selenium levels and sources and dietary fat quality in diets for broilers breeders and layer hens. Poultry Science 87:2605-2612.
9. O'Grady M.N.; Monahan F.J.; Fallon R.J.; Allen P. (2001). Effects of dietary supplementation with vitamin E and organic selenium on the oxidative stability of beef. J.Animal Sci. 79:2827-2834.
10. Barnes K.M., Evenson J.K., Raines A.M., Sunde R.A. (2009). Transcript analysis of the selenoproteome indicates that dietary selenium requirements of rats based on selenium-regulated selenoprotein mRNA levels are uniformly less than those based on glutathione peroxidase activity. J. Nutr. 139:199-206.
11. Mahan D.C; Cline T.R.; Richert B. (1999). Effects of dietary levels of Selenium-enriched yeast and sodium selenite as selenium sources fed to growing-finishing pigs on performance, tissue selenium, serum Glutathione Peroxidase activity, carcass characteristics, and loin quality. J. Anim. Sci. 77:2172-2179.
12. Payne R.L., Southern L.L. (2005). Comparison of inorganic and organic selenium sources for broilers. Poultry Science 84:898-902.
13. Juniper D.T.; Phipps R.H.; Ramos-Morales E.; Bertin G. (2008). Effect of dietary supplementation with selenium-enriched yeast or sodium selenite on selenium tissue distribution and meat quality in beef cattle. J. Anim. Sci. 86:3100-3109.
14. Bunk M. J., Combs G. F. (1981). Relationship of selenium-dependent glutathione peroxidase activity and nutritional pancreatic atrophy in selenium-deficient chicks. J. Nutr. 111, 1611-1620.

ROSEMARY EXTRACT EFFECT ON FRESH SAUSAGES PRESERVATION TO REPLACE SULPHITES

R. Teruel, M. Egea, M. Mariella, M.D. Garrido, M.B. Linares*

Department of Food Technology, Nutrition and Bromatology, Veterinary Faculty, University of Murcia, Espinardo, 30071 Murcia, Spain

*blinares@um.es

Abstract – The aim of this study was to evaluate the effect of rosemary extract on fresh sausages quality stored 15 days at 4 ºC in order to eliminate the sulphites from the formulation. Three treatment were assigned: Sulphites (SU), Rosemary (RO) and Sulphites + Rosemary (SU+RO). Microbiological determinations (total viable count), lipid oxidation and colour (CIELab coordinates) were evaluated. Rosemary addition did not significantly vary the microbial growth in samples being the lowest microbial counts in SU group. For the RO group not significant differences were described along storage for the lipid oxidation process. SU and SU+RO group showed the most intense antioxidative effect after 5 days storage. L* and b* were not affected by treatment or storage. The a* coordinate was more stable in both groups that contained sulphites. In conclusion, rosemary extract at 120 ppm dose used in the present study was not enough to replace sulphites effect as respect colour and microbiological counts, but higher oxidative stability was described in rosemary samples group (RO).

I. INTRODUCTION

Although the use synthetic additives to control spoilage changes in food have been highly considered in meat product, at present, there is a tendency to restrict their use (1). This is due, in part, to the adverse reactions described in sensitive individuals after consuming certain foodstuffs i.e. those contain sulfites (2). The increase of concern on consumer health involved the refusing to use synthetic additives, with the incorporation of natural additives to extend shelf life and/or improve food safety (3). Rosemary (*Rosmarinus officinalis* L.) extracts have exhibited potent antioxidant activity and are widely used in the food industry (4). Lipid oxidation, which translates to rancidity, is one of the major reasons of meat products' quality deterioration. (3) that contributes to the development of unacceptable organoleptic characteristics (3), what is especially important because appearance of meat products is one of the major determinants to consider by consumers at purchasing time (5). For all these reasons, the aim of this research was to investigate the use of rosemary extracts to improve oxidative and sensory stability on two meat products manufactured without any artificial additive in fresh sausages.

II. MATERIALS AND METHODS

A powder rosemary extract, was used for fresh sausages elaborations (174.2 g/kg carnosic acid and 28.4 g/kg carnosol). Pork sausages were formulated with 40% fat and 60% meat. The meat was blended with a commercial mixture based on: salt, dextrose, vegetable fiber, spices, starch, corn dextrin, preservative E-224, antioxidant E-301 and colorant E-120 (40 g/kg meat). There were three treatment groups: SU─(control, with 450 ppm of sulphites), RO (without sulphites with 120 ppm of rosemary extract) and SU + RO (with 450 ppm of sulphites and 120 ppm of rosemary extracts). The meat was minced (5 mm) using a P3298 cutter (Braher International, San Sebastian, Spain). All ingredients were homogenized and then were stuffed into collagen casings, weighting 40 g each sample. The samples were packaged in clear trays of crystal polystyrene (B5-37 aerpack, ALIGA and Ortiz. SL, Spain), over-wrapped with a permeable film (MICAL® professional, Miquel Alimentació Group S.A.U, Vilamalla, Spain.) and stored for 15 days at 4 °C in a display cabinet illuminated with white fluorescent light (620 lux) simulating retail display conditions. Two packs were opened for subsequent analysis for each

treatment on days 0, 5, 9 and 15 of storage. The entire experiment was duplicated.

Total viable counts (TVC) was determined by pour plate methods in Plate Count Agar, using conventional dilution procedures. The plates were incubated for 48 h at 37 ºC. Lipid oxidation was assessed through determination Malondialdehyde (MDA) content in accordance to described by Botsoglou *et al.* (6). Colour was measured using a CR-400 Chroma Meter (Minolta Ltd., Milton Keynes, United Kingdom) calibrated against a standard white tile (8 mm diameter aperture, d/0 illumination system, D65 illuminant and a 2º standard observer angle). All data were analysed by using the SPSS.19 statistical package.

III. RESULTS AND DISCUSSION

Table 1 Quality parameters from fresh sausages with rosemary extract stored during 15 day at 4°C

	Storage time (day)	Treatment SU	ROS	SU + ROS	
Total viable counts	0	5.9±0.2	5.9±0.9	5.1±0.5	NS
	5	4.8±0.6^{ab}	6.2±0.2 ^{b}	4.7±0.1^{a}	*
	9	4.7±0.2^{a}	7.1±0.1 ^{b}	4.8±0.1 ^{a}	**
	15	4.6±0.2	6.9±0.6	5.8±2.0	NS
		NS	NS	NS	
TBARS	0	0.1±0.18^{ax}	0.0±0.0^{ab}	0.1±0.0^{ax}	NS
	5	0.3±0.0^{bxy}	0.1±0.1^{a}	0.0±0.0^{ax}	**
	9	0.3±0.02^{xy}	0.2±0.1	0.2±0.0^{xy}	NS
	15	0.4±0.1 ^{y}	0.3±0.1	0.4±0.2^{y}	NS
		*	NS	*	

NS: no significative; *: P<0.05; **: P<0.01 ;***:0.001; a. b: treatments group effect. x. y: storage effect.

Table 1 shows the quality parameters for the different sausages batches. The TVC values were higher for RO group than SU and SU + RO groups (P<0.01), on day 5 and 9, reaching higher levels than established by EU legislation 1141/2007. Although rosemary has non-polar components such as phenolic diterpenes, responsible for the antimicrobial properties (3), the antibacterial effect of rosemary was lower to that of sulphites. In contrast, a previous study in fresh chicken sausages (3) described a light reduction of total viable count when rosemary was added at 500, 1000 and 1500 ppm, however in this study authors did not add sulphites but rosemary extracts doses were higher than in the present research.

Table 2 Colour parameters from fresh sausages with rosemary extract stored during 15 day at 4°C

	Storage time (day)	Treatment SU	ROS	SU + ROS	
L*	0	46.0±2.2^{x}	44.6±3.5^{x}	43.2±3.7	NS
	5	51.2±1.5^{xy}	45.9±4.0	44.3±1.4	NS
	9	54.2±1.8^{y}	53.1±2.7^{y}	54.1±5.3	NS
	15	50.4±4.6^{axy}	45.0±0.9^{abx}	43.7±1.4^{b}	*
		*	***	NS	
a*	0	20.9±0.6^{abx}	19.0±1.3^{ax}	22.2±1.3^{b}	**
	5	18.5±1.3^{axy}	17.6±1.1^{ax}	21.1±0.9^{b}	**
	9	23.1±2.0 ^{by}	17.4±0.7^{ax}	21.9±1.5^{b}	**
	15	18.8±1.9^{axy}	13.1±0.9^{by}	21.0±2.1^{a}	***
		**	**	NS	
b*	0	5.4±0.4	5.7±0.9	5.8±0.7	NS
	5	5.6±0.1	5.3±0.6	5.2±0.5	NS
	9	7.4±1.2	5.9±1.0	6.3±1.0	NS
	15	5.1±1.2	4.3±0.5	5.6±0.8	NS
		NS	NS	NS	

NS: no significative; *: P<0.05; **: P<0.01 ;***:0.001; a. b: treatments group effect. x. y: storage effect.

In general there were not variations as respect the MDA levels in any of the groups by storage time, although a higher oxidative stability was described for RO samples by time. Oxidation stability was higher for RO and SU+RO groups during 5 days of storage. At the end of the storage, all the groups had similar values of MDA content (P>0.05). It is probably that both antioxidants, ascorbic acid (included in the commercial formula) and rosemary extracts, had enough capacity to prevent the rancidity processes development. Lee *et al.* (7) evaluated the oxidative stability in chicken sausages obtained similar results. These authors also based their result on the presence of substance with antioxidants activity in the formulation.

For the L* and b* coordinates not significant differences between treatments groups were observed along storage (Table

2). The a* coordinate was lower in RO group than SU and SU+RO group. In addition, both groups with sulphites added manintaned the colour at the end of storage. Similar results were found by Sebranek *et al.* (8) in fresh refrigerated pork sausage elaborated with different rosemary concentration.

Therefore It can be concluded that the only addition of rosemary extracts could not to preserve the colour stability of samples during storage.

IV. CONCLUSION

Rosemary extracts at 120 ppm used in the product in absence of sulphites could maintain the oxidative stability along storage but had not showed any effect on colour protection and spoilage development. For that reason the rosemary extract dose used in the present study was not enough to replace sulphites in fresh pork sausage stored during 15 days.

REFERENCES

1. Decker, E. A., & Mei, L. (1996). Antioxidants mechanisms and application in muscle food. Poccedings of the Reciprocal Meat Conference 49: 64-72.
2. Peña-Egido, M. J., García-Alonso, B., & García-Moreno, C. (2005) S-Sulfonate Contents in Raw and Cooked Meat Products. Journal of Agriculture and Food Chemistry 53: 4198-4201.
3. Liu, D. C., Tsau, R. T., Lin, Y. C., Jan, S. S., & Tan, F. J. (2009). Effect of various levels of rosemary or Chinese mahogany on the quality of fresh chicken sausage during refrigerated storage. Food Chemistry 117(1): 106-113.
4. Basaga. H., Tekkaya. C., & Acitel. F. (1997). Antioxidative and free radical scavenging properties of rosemary extract. Lebensmittel – Wissenschaft und Technologie 30(1): 105–108.
5. Clydesdale. F. M. (1998). Color: origin. stability. measurement and quality. In A. T. Irwin & R. P. Singh (Eds.). Food storage stability (pp. 175–190). Florida: CRC Press. Boca Raton.
6. Botsoglou, N. A., Fletouris, D. J., Papageorgiou, G. E., Vassilopoulos, V. N., Mantis, A. J., & Trakatellis, A. G. (1994) A rapid sensitive and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissues. food. and feedstuff samples. Journal of Agriculture and Food Chemistry 42: 1931–1937.
7. Lee, T. G., Williams, S. K., Solan, D., & Littell, R. (1997) Development and evaluation of a chicken breakfast sausage manufactured with mechanically deboned chicken meat. Poultry Science 76:415-421.
8. Sebranek, J. G., Sewalt, V. J. H., Robbins, K. L., & Houser, T. A. (2005) Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat Science 69:289-296.