American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

American Journal of Biochemistry and Molecular Biology 3 (2): 240-247, 2013
ISSN 2150-4210 / DOI: 10.3923/ajbmb.2013.240.247
© 2013 Academic Journals Inc.

Ultrasonication of Chicken Natural Actomyosin: Effect on ATPase Activity, Turbidity and SDS-PAGE Profiles at Different Protein Concentrations

Riaz Ahmad and Absar-ul Hasnain
Department of Zoology, Section of Genetics, Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India

Corresponding Author: Absar-ul Hasnain, Department of Zoology, Section of Genetics, Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India Tel: +91-571-2700920/3730

ABSTRACT

With the increasing application of ultrasonics in meat tenderization and processing, physicochemical events initiated by sonic radiation at myofibrillar level and propagated in complex tissue such as meat require a clear understanding. The enormous amount of basic information collected by studying myofibrils, actomyosin or their individual constituents has already clarified intricacies of muscular contraction and, part of such basic information has find application in meat sciences. In this investigation, chicken Natural actomyosin (NAM) has been taken as a simple model to work out some effects of ultrasonication in a concentration range of 0.5 to 1.8 mg protein mL^{-1}. At each concentration, NAM solution in 0.6 M NaCl (2.0 mL) was individually exposed to 20 kHz sonic waves for a total of 10 min. Cooling was maintained by keeping NAM containers in crushed ice and a lag of 5 sec after each 10 sec long sonic burst. Aliquots from each sonicated NAM were subjected to biochemical analyses. Most striking differences were observed in Ca^{2+}-ATPase activity, which displayed a steady decline that corresponded with the decreasing protein concentration. Ultrasonication of NAM for 10 min caused a loss of ~47% of Ca^{2+}-ATPase activity at the highest dilution (0.5 mg mL^{-1}). In the same order of protein concentration, turbidity of ultrasonicated NAM also decreased which denotes increasing transparency. Thus, ATPase and turbidity data demonstrate that due to sonic radiation, interactions among constituents of chicken actomyosin complex alter and these structural changes are devoid of any fragmentation. Under present experimental conditions, SDS-PAGE profiles did not reveal any novel band which could be attributed to ultrasonic fragmentation or proteolytic contamination. The findings also suggest that unlike myofibrils, actomyosin is a model where interactions and substructural changes of constituent polypeptides can be investigated without interference of endogenous muscle proteases.

Key words: ATPase activity, natural actomyosin, SDS-PAGE, turbidity profiles, ultrasonication

INTRODUCTION

High intensity ultrasonication is getting increasing application in various food technologies and several reviews are available on this subject (Dolatowski *et al*., 2007; Chemat *et al*., 2011). In meat industry, sonic radiation is being used for curing (Siro *et al*., 2009) and tenderizing carcass meat (Lyng *et al*., 1997; Got *et al*., 1999; Jayasooriya *et al*., 2004). The impact of ultrasonication on meat involves generation of heat by pressure waves, changes in pH or color and water holding capacity as well as substructural disorganization (Latoch, 2010). Even fragmentation or solubilization of

some of the structural proteins might take place in majority of meat types (Ito *et al*., 2003; Stadnik *et al*., 2008). At high intensity sonic radiation, microstructure of collagen fibers might get severely altered and products be spilled over to occupy intracellular spaces (Chang *et al*., 2012). Aging and endogenous proteases, released during lysosomal disruption, are inherent participants in tenderization process attributed to fragmentation of various structural proteins (Jayasooriya *et al*., 2007).

A problem associated with meat of higher vertebrates is the substantial amount of collagen network. Chicken meat has quite low collagen contents and chicken myofibrillar proteins have been successfully solubilized by ultrasonication at 20 kHz for 10 min (Ito *et al*., 2004). Natural actomyosin (NAM) is the contractile complex that constitutes bulk of muscle mass extractable in high salt solutions, either directly from muscle mince or myofibrillar preparations. SDS-PAGE profiles of chicken NAM have so far revealed no contamination originating from collagen. NAM thus represents the principal contractile mass of myofibrillar proteins *in vitro*. Because of the above mentioned advantages, chicken Natural actomyosin (NAM) has been chosen here as the model to initially investigate some of the biochemical events after exposure to 20 kHz sonic radiation for 10 min. Virtually no information has been published on isolated NAM till now. The investigation takes in to account protein concentration dependence as the variable of the sonication-induced changes in Ca^{2+}-ATPase, turbidity and SDS-PAGE profiles. The selected parameters reflect changes in actin-myosin interaction, dissociability or aggregation of the complex and substructural variations.

MATERIALS AND METHODS

Location of studies and design of research plan: The experiments were carried out at Zoology Department of Aligarh Muslim University, Aligarh (Uttar Pradesh, India) during the last one year. The proposal originated from a need to elucidate biochemical events at the basic level of organization of contractile complex (natural actomyosin, NAM) without interference from lysosomal proteases and other myofibrillar proteins of chicken skeletal muscle. It was envisaged that Ca^{2+} activated ATPase, turbidity measurements and polypeptide composition on SDS-PAGE profiles will provide selective information on sonic radiation-induced changes in chicken NAM, in terms of actin-myosin interaction and structural changes or fragmentation.

Source of chemicals: All the chemicals and reagents used in this study were of analytical grade. Acrylamide, bis-acrylamide, Phenyl methane sulfonylfluoride (PMSF), Adenosine 5'-triphosphate (ATP) disodium salt, ammonium per sulphate and TEMED were procured from authorized dealers of Sigma-Aldrich Chemicals Pvt. Ltd. (USA) in India. Sodium chloride, 1-amino-2-naphthol-4-sulphonic acid, bovine serum albumin, Tris buffer and all other reagents were purchased from SRL Chemicals, India.

Source of animal and muscle type: *Pectoralis major* from the breast muscle mass of the broilers of 3 month age were dissected out post-sacrifice and immersed in ice bath. Muscle was chopped finely and washed 3 times with several volumes of 5 mM phosphate buffer (pH 7.0) containing 2 mM PMSF before proceeding for actomyosin extraction.

Extraction and purification of actomyosin: Natural actomyosin (NAM) from the washed pellet obtained as above was extracted as described previously (Ahmad and Hasnain, 2006;

Hasnain and Ahmad, 2006) with additional step of eliminating contamination of free myosin. Briefly, the pellet of muscle mince was suspended in extraction buffer (0.45 M KCl containing 25 mM phosphate buffer, pH 7.0). After gentle mixing and overnight storage under crushed ice, viscous NAM was centrifuged at 10 K rpm (4°C) and precipitated by 10-fold dilution with chilled distilled water. Pellet was saved and dissolved in solvent buffer (0.6 M NaCl with 20 mM Tris-maleate, pH 7.0). Traces of free myosin were removed by washing two times with 0.2 M NaCl. It was followed by two cycles of dissolving NAM pellet in solvent buffer and precipitation with distilled water to 0.06 M NaCl, to eliminate trace low ionic strength salt soluble impurities. Finally, NAM dissolved in solvent buffer was dialyzed overnight against the solvent buffer, cleared by centrifugation at 10 K rpm (4°C) and stored under crushed ice during investigations which were completed within 24 h.

Protein estimation: Protein concentration was determined by Biuret method of Gornall *et al.* (1949) using bovine serum albumin as standard. Three dilutions (0.5, 1.3 and 1.8 mg mL^{-1}) of NAM were subjected to ultrasonication and subsequent biochemical analyses. All dilutions were made in NAM solvent buffer (0.6 M NaCl with 20 mM Tris-maleate, pH 7.0).

Ultrasonic treatment: Ultrasonication was performed using Ralco immersible-probe ultrasonicator for a total of 10 min at 20 kHz. During ultrasonic treatment, the NAM containing glass beaker was kept in ice bath intervened by cooling lags of 5 sec after each 10 sec of ultrasonic burst. The duration of 10 min is the sum of only the ultrasonication bursts of 10 sec and does not include cooling intervals of 5 sec. Samples were subjected to biochemical analyses immediately.

Ca^{2+}-ATPase assay and electrophoretic profiling: Ca^{2+}-ATPase was assayed at 20°C in final concentrations of 50 mM NaCl, 20 mM Tris-maleate of pH 7.0, 5 mM $CaCl_2$ and 1 mM ATP and, the liberated Pi measured as reported by Hasnain *et al.* (1979). Turbidity was monitored at 340 nm using UV-VIS 118 Spectrophotometer (Systronics). SDS-PAGE in 12% gels was performed essentially as described previously (Ahmad *et al.*, 2012). Following overnight fixing and washing in 10% acetic acid-5% methanol, protein bands were visualized by staining with Coomassie Brilliant Blue R-250. Background was cleared by washing in 7% acetic acid.

Documentation and densitometry: Stained SDS PA-gels were documented at different contrasts using digital camera (SONYCYBERSHOT: Zoom-4X, 12 Megapixels) and by scanning on an all-in-one HP Deskjet (F370) computer setup. Densitometry of the gel-lanes was carried out using Scion Imaging (Scion Corporation; Beta release, 4.0) and GelPro (Media Cybernetics, USA) software programs.

RESULTS

Figure 1 demonstrates the effect of ultrasonication (20 kHz) on chicken NAM at the three concentrations. In comparison with the control (unsonicated NAM), sonication for 10 min resulted in a loss of ~23%, ~19% and ~14% Ca^{2+}-ATPase activity at protein concentrations of 1.8, 1.3 and 0.5, respectively. Turbidity measurements at these NAM concentrations showed a decline of 10, 25, 50%, respectively, indicating increasing transparency subsequent to sonication (Fig. 2).

SDS-PAGE profiles of ultrasonicated chicken NAM along with the control are shown in Fig. 3a. Each lane displays the same number of polypeptides with characteristic molecular

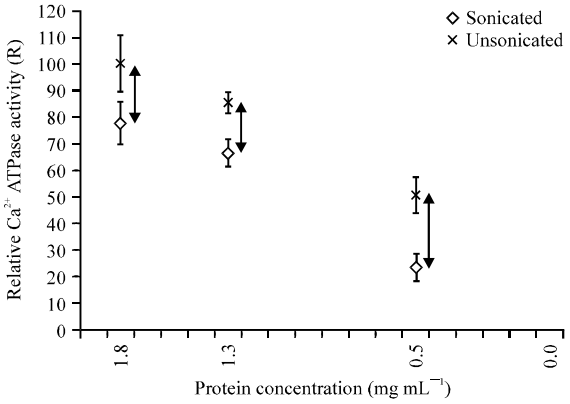

Fig. 1: Protein concentration dependence of ultrasonication on Ca^{2+}-ATPase activity of chicken natural actomyosin (NAM), Unsonicated NAM has been taken as the control

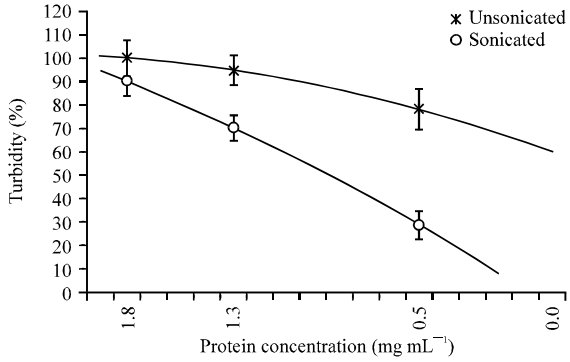

Fig. 2: Sonication induced decrease in turbidity of chicken natural actomyosin (NAM) as a function of decreasing protein concentration

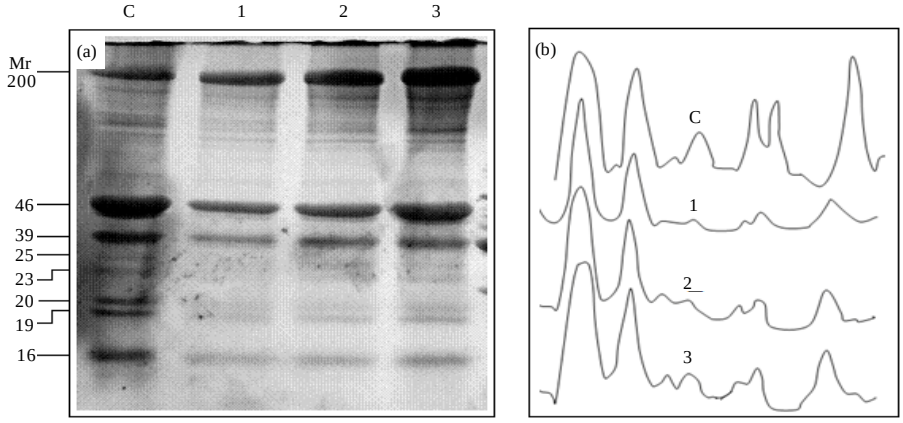

Fig. 3(a-b): (a) SDS-PAGE profiles of chicken natural actomyosin (NAM) control and sonicated dilutions, Chicken control polypeptides were taken as the markers, Established molecular weights (M_r) of myosin heavy chain, actin, troponin-I, tropomyosin and light chains are labelled on the left of the profiles, (b) Densitometric tracings of the four lanes of Fig. 3a in the same order, The scans display only those polypeptides which stack below actin

weights of 200, 46 and 39 kDa for myosin heavy chain, actin and tropomyosin, respectively. Polypeptides below tropomyosin are troponin-I (23 kDa) and troponin-C (19 kDa) and the rest (25, 20 and 16 kDa) myosin light chains. No novel band was visualized. The relative intensities of these bands correspond to the increasing concentrations of NAM from 0.5 to 1.8 mg mL^{-1}. Densitometry of the polypeptides stacking below the actin (46 kDa band) also does not reveal the presence of any new band and accords with the protein dilutions (Fig. 3b). Scan of the portion of gels above the actin band was kinky due to background and has, therefore, been excluded. However, as the Fig. 3 shows there is no apparent cleavage in the main band of myosin heavy chain.

DISCUSSION

Reports which described fragmentation of isolated myosin and heavy meromyosin (HMM) by ultrasonication are rather old (Barany *et al*., 1963a, b). Isolated actin, the other integral protein component of actomyosin complex is a thin filament protein of myofibrils and its G-monomer does not fragment under sonication (Asakura, 1961; Asakura *et al*., 1963). Its fibrous polymer (F-actin) fragments under ultrasonic influence; but, the fragments polymerize into larger than the usual polymers (Asakura *et al*., 1963; Carlier *et al*., 1985). In an earlier study, ATP hydrolysis by actin and the polymerization was shown to display veritable responses to millimolar vs. micromolar concentrations of calcium (Dancker and Low, 1977). Anyhow, the above literature deals with isolated actin and not as a constituent of natural actomyosin complex. Whereas even F-actin polymer will stack as a single band in SDS-PAGE due to dissociation by SDS, fragmentation of myosin can be reliably monitored by this technique, though the number and intensity of novel bands depend on a multitude of factors. Another problem with conventionally prepared tryptic subfragments of myosin (e.g., HMM) is that they are already heterogenous in gel profiles (Samejima *et al*., 1976). More stable and homogenous myosin subfragments are generated by other enzymes and chemical cleavages (Samejima *et al*., 1981).

Therefore, with the objective to understand the basics of the mechanism of ultrasonic effects, natural actomyosin was chosen as a model that emulates conditions within the myofibrils. Actomyosin is the contractile proteins complex that constitutes the bulk of myofibrillar proteins and muscle mass. It was perceived that Natural actomyosin (NAM) is a model wherein the impact of ultrasonication can be directly observed in terms of ATPase activities, interactions of actin with myosin along with regulatory proteins (troponins and tropomyosin) or overall aggregation, fragmentation or dissolution behavior. As huge amount of literature suggests, SDS-PAGE provides a direct look into fragmentation and several of the events related with the above aspects. Use of actomyosin also eliminates fragmentation of myosin and associated proteins by intrinsic proteases which may be released or activated post mortem (Koohmaraie and Geesink, 2006; Goll *et al*., 2008). The use of fresh actomyosin is also recommended, since myosin heavy chain may get fragmented even in highly purified myosin preparation when aged and kept at warm temperature (Hasnain, 1984).

The results of this study (Fig. 1) demonstrate that after 10 min of ultrasonication and irrespective of the nature of other changes in NAM, Ca^{2+}-ATPase declines. The data also suggests that the magnitude of ultrasonication effect would be protein concentration dependent and, therefore, protein concentration variables should be carefully considered for a meaningful comparative study. An initial activation of Ca^{2+}-ATPase of avian myofibrils was reported by Talesara and Narang (1977), however, under present conditions no such activation was observed

for chicken NAM. No fragmentation was visualized in SDS-PAGE profiles also, that is in agreement with those obtained even on meat slices (Lyng *et al*., 1997, 1998). However, a clearing effect, as apparent by reduction in turbidity, suggests change in interactions of NAM constituents, in particular actin-myosin interaction as initially suggested by decline in Ca^{2+}-ATPase activity.

Since conformation of actin as well as myosin undergoes changes under certain conditions (Asakura *et al*., 1963; Hayakawa *et al*., 2010), the state of either of these proteins can potentially alter the actin-myosin interaction. The later authors have shown that myosin can be made soluble at low ionic strength solutions instead of normal 0.2 M salts (Hayakawa *et al*., 2009). More importantly, the conformation dependent size variation within a portion of myosin rod can depolymerize myosin filaments (Hayakawa *et al*., 2010). The important role played by myosin tail domain has been emphasized in non-muscle myosin, as well (Guthrie, 2012). It is well established that subsequent to mixing myosin with actin, an abrupt increase in turbidity occurs due to actomyosin complex formation and the salt concentration to keep actomyosin in solution is 0.6 M KCl. In contrast, individual preparations of actin as well as myosin are transparent and have lower salt solubility characteristics. Therefore, a conformation dependent change in the solubility profiles of actin and myosin will eventually affect the nature of actomyosin complex and, in turn, ATPase and other properties of such a complex. It is likely that some of the changes in interaction of actomyosin (cell-free system) and myofibrillar proteins (myofibril-trapped actomyosin) due to ultrasonic effect may be common in some of details; but they may differ in several other respects, such as the action and consequences of intracellular proteases.

CONCLUSIONS

The results of this study suggest that sonication of chicken Natural actomyosin (NAM) at 20 kHz for 10 min causes no fragmentation of constituent polypeptides. Rather a reduction in actin-myosin interaction (dissociation) occurs that is evident from reduced turbidity which is a measure of increasing transparency. The remarkable decline in Ca^{2+}-ATPase appears to be the consequence of actin-myosin dissociation. The findings support use of actomyosin as a simple cell-free model to follow ultrasonication-induced changes which initiate in the contractile apparatus and subsequently affect state of muscle. The change in investigated properties is protein concentration dependent, suggesting caution in comparative studies.

ACKNOWLEDGMENTS

Corresponding author is grateful to the University Grants Commission (UGC), New Delhi for funding this work. Authors are obliged to the University and the Chairman, Department of Zoology for laboratory facilities and to Prof. Abbas Abidi for his help. We also acknowledge with thanks the technical support by Miss Areeba Ahmad.

REFERENCES

Ahmad, R. and A. Hasnain, 2006. Correlation between biochemical properties and adaptive diversity of skeletal muscle myofibrils and myosin of some air-breathing teleosts. Ind. J. Biochem. Biophys., 43: 217-225.

Ahmad, R., R.B. Pandey, S.H. Arif, N. Nabi, M. Jabeen and A. Hasnain, 2012. Polymorphic β and γ lens crystallins demonstrate latitudinal distribution of threatened walking catfish *Clarias batrachus* (Linn.) populations in North-western India. J. Biol. Sci., 12: 98-104.

Asakura, S., 1961. F-actin adenotriphosphate activated under sonic vibration. Biochim. Biophys. Acta, 52: 65-75.

Asakura, S., M. Taniguchi and F. Oosawa, 1963. Mechano-chemical behaviour of F-actin. J. Mol. Biol., 7: 55-69.

Barany, K., M. Barany, H. Oppenheimer and T.T. Antony, 1963a. Effects of ultrasonics on the size and shape of L-myosin and the meromyosins. Biochim. Biophys. Acta, 74: 551-554.

Barany, M., K. Barany and H. Oppenheimer, 1963b. Effect of ultrasonics on the adenotriphosphate activity and actin binding ability of L-myosin and heavy meromyosins. Nature, 199: 694-695.

Carlier, M.F., D. Pantaloni and E.D. Korn, 1985. Polymerization of ADP-actin and ATP-actin under sonication and characteristics of the ATP-actin equilibrium polymer. J. Biol. Chem., 260: 6565-6571.

Chang, H.J., X.L. Xu, G.H. Zhou, C.B. Li and M. Huang, 2012. Effects of characteristics changes of collagen on meat physicochemical properties of beef semitendinosus muscle during ultrasonic processing. Food Bioprocess Technol., 5: 285-297.

Chemat, F., Z. Huma and M.K. Khan, 2011. Application of ultrasound in food technology: Processing, preservation and extraction. Ultrason. Sonochem., 18: 813-835.

Dancker, P. and I. Low, 1977. Dual effect of Ca^{2+} on ultrasonic ATPase activity and polymerization of muscle actin. Biochim. Biophys. Acta, 484: 169-176.

Dolatowski, Z.J., J. Stadnik and D. Stasiak, 2007. Applications of ultrasound in food technology. Acta Sci. Pol. Technol. Aliment, 6: 89-99.

Goll, D.E., G. Neti, S.W. Mares and V.F. Thompson, 2008. Myofibrillar protein turnover: The proteasome and the calpains. J. Anim. Sci., 86: E19-E35.

Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of biuret reaction. J. Biol. Chem., 177: 751-766.

Got, F., J. Culioli, P. Berge, X. Vignon, T. Astruc, J.M. Quideau and M. Lethiecq, 1999. Effects of high-intensity high-frequency ultrasound on ageing rate, ultrastructure and some physico-chemical properties of beef. Meat Sci., 51: 35-42.

Guthrie, O.W., 2012. Nuclear association of nonmuscle myosin-II within the giant cells of *Drosophila melanogaster* salivary gland organ: Tail domain specifies perinuclear oligomerization. Am. J. Biochem. Mol. Biol., 2: 30-39.

Hasnain, A. and R. Ahmad, 2006. A study on some biochemical properties of active subfragment-1(S1) prepared from actomyosin of air-breathing freshwater teleost, *Channa gachua*. Biomed. Res., 17: 27-31.

Hasnain, A., 1984. Proteolytic contamination of carp myosin preparation revealed by thermal treatment. Ind. J. Exp. Biol., 22: 172-173.

Hasnain, A., K. Samejima, K. Takahashi and T. Yasui, 1979. Molecular adaptability of carp myosin: A study of some physic-chemical properties and their comparison with those of rabbit myosin. Arch. Physiol. Biochem., 87: 643-662.

Hayakawa, T., T. Ito, J. Wakamatsu, T. Nishimura and A. Hattori, 2009. Myosin is solubilized in aneutral and low ionic strength solution containing L-histidine. Meat Sci., 82: 151-154.

Hayakawa, T., T. Ito, J. Wakamatsu, T. Nishimura and A. Hattori, 2010. Myosin filament depolymerizes in a low ionic strength solution containing L-histidine. Meat Sci., 84: 742-746.

Ito, Y., R. Tatsumi, J.I. Wakamatsu, T. Nishimura and A. Hattori, 2003. The solubilization of myofibrillar proteins of vertebrate skeletal muscle in water. Anim. Sci. J., 74: 417-425.

Ito, Y., S. Toki, T. Omori, H. Ide and R. Tatsumi *et al.*, 2004. Physicochemical properties of water soluble myofibrillar proteins prepared from chicken breast muscle. Anim. Sci. J., 75: 59-65.

Jayasooriya, S.D., B.R. Bhandari, P. Torley, B.R. D'Arcy, 2004. Effect of high power ultrasound waves on properties of meat: A review. Int. J. Food Prop., 7: 301-319.

Jayasooriya, S.D., P.J. Torley, B.R. D'Arcy and B.R. Bhandari, 2007. Effect of high power ultrasound and ageing on the physical properties of bovine *Semitendinosus* and *Longissimus* muscles. Meat Sci., 75: 628-639.

Koohmaraie, M. and G.H. Geesink, 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Sci., 74: 34-43.

Latoch, A., 2010. Selected properties of I-Z-I properties of sonicated beef. Pol. J. Food Nutr. Sci., 60: 51-55.

Lyng, J.G., P. Allen and B. McKenna, 1998. The effects of pre and post-rigor high-intensity ultrasound treatment on aspects of lamb tenderness. LWT-Food Sci. Techn., 31: 334-338.

Lyng, J.G., P. Allen and B.M. McKenna, 1997. The influence of high intensity ultrasound baths on aspects of beef tenderness. J. Muscle Foods, 8: 237-249.

Samejima, K., K. Takahashi and T. Yasui, 1976. Heat induced gelation of myosin total rod. Agric. Biol. Chem., 40: 2455-2464.

Samejima, K., M. Ishioroshi and T. Yasui, 1981. Relative roles of the head and tail portions of the molecule in heat-induced gelation of myosin. J. Food Sci., 46: 1412-1418.

Siro, I., C. Ven, C. Balla, G. Jonas, I. Zeke and L. Friedrich, 2009. Application of an ultrasonic assisted curing technique for improving the diffusion of sodium chloride in porcine meat. J. Food Eng., 91: 353-362.

Stadnik, J., Z.J. Dolatowski and H.M. Baranowska, 2008. Effect of ultrasound treatment on water properties and microstructure of beef (*M. semimembranous*) during ageing. LWT-Food Sci. Technol., 41: 2151-2158.

Talesara, C.L. and V. Narang, 1977. Effects of ultrasonic, storage and freeze-thawing on the myofibrillar ATPase activity of skeletal muscles. Curr. Sci., 46: 861-862.