International Food Research Journal 21(5): 2049-2054 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of ammonium hydroxide on textural and ultrastructural properties of spent hen meat

[1*]Kiran, M., [2]Naveena, B. M., [1]Sudhakar Reddy, K., [1]Kondal Reddy, K. and [3]Madhav Rao, T.

[1]*Department of Livestock Products Technology, College of Veterinary Sciences, Hyderabad, 500030, India*
[2]*National Research Centre on Meat, Chengicherla, Hyderabad, 500092, India*
[3]*Department of Veterinary Public Health, College of Veterinary Sciences, Hyderabad, 500030, India*

Article history

Received: 10 November 2012
Received in revised form: 30 March 2014
Accepted: 1 April 2014

Keywords

Spent hen meat
Tenderness/texture
Ammonium hydroxide
Collagen solubility
Ultrastructure

Abstract

Beneficial effects of ammonium hydroxide was studied, to optimize the ammonium hydroxide concentration for effective tenderization of spent hen meat. Spent hen meat chunks were subjected to ammonium hydroxide treatment (AHT) at different concentrations (0%, 0.1%, 0.5% and 1.0% v/w) and evaluated for different quality parameters after 24 hours. The results indicated a significant increase in pH, water holding capacity, total and myofibrillar protein solubility, collagen solubility in 0.5% and 1.0% AHT samples relative to control with significant reduction in Warner-Bratzler shear force (WBSF) for all AHT meat chunks. The SDS-PAGE photographs also revealed a reduction in the band colour intensity in all AHT samples compared to control indicating breakdown of proteins. Transmission electron microscopy (TEM) also confirms the proteolysis and breakdown of muscle fibres in AHT samples. These results clearly suggest the tenderizing effects of ammonium hydroxide in spent hen meat. There was no significant improvement in tenderness at 1% level compared to 0.5% level, therefore ammonium hydroxide at 0.5% was suggested as the optimal for the spent hen chicken tenderization.

Introduction

Poultry meat accounts for 30% of global meat consumption and by 2020 poultry is predicted to become the overall meat of choice (Bilgili, 2002). In recent years, the poultry industry has experienced an unrivalled growth rate. Spent hen meat is very tough, and this toughness prevents its use as whole-muscle food products and reduces its market value (Nurmahmudi and Sams, 1997). Large number of broiler and layer parent stocks, spent and culled hens will be available due to fast growing layer industry around the World. Disposal of this large chunk of birds will be a major problem for poultry industry (Naveena *et al.*, 2012). Spent hen meat, due to some of the inherent reasons is tough and difficult to process. Because of unacceptable toughness and brittle bones, the use of spent hen meat has long been a problem for the poultry industry. If tenderness could be improved it would be possible to expand the market for the spent hen meat and increase its value. Ammonium hydroxide is listed as generally regarded as safe (GRAS) by Food and Drug Administration (FDA, USA) (21 CFR 184.1139) with no limitation other than current good manufacturing practices for uses as leavening agent, pH control agent, surface finishing agent, boiler water additive, and food additive. Ammonium hydroxide is a food additive that is included in Table 3 of GSFA (General Safety for Food Adulterants), and as such may be used in many foods under the conditions of good manufacturing practices (GMP) as outlined in the Preamble of the Codex GSFA (General Safety for Food Adulterants; GSFA, 2009). Beneficial effects of ammonium hydroxide in beef steaks in improving shear force value, tenderness, and sensory traits are reported by few researchers (Hamling and Calkins, 2008, Hamling *et al*., 2008). Significant improvement in myoglobin redox stability, cooking yield, water holding capacity, instrumental colour and a significant reduction in lipid oxidation of ground buffalo meat treated with ammonium hydroxide was recently reported by Naveena *et al.* (2011a). Naveena *et al*. (2011b) have also reported a significant improvement in tenderness of buffalo meat chunks treated with ammonium hydroxide. Even though, few unpublished reports and international patents reveal the multifunctional uses of ammonium hydroxide in meat and meat products, no systematic studies on their effect on different quality attributes of meat and meat products are reported. Hence, this study was undertaken to evaluate the effect of ammonium hydroxide on textural, physicochemical, and ultrastructural properties of spent hen meat and also to determine optimum concentration required to

*Corresponding author.
Email: *kiranm.321@rediffmail.com*
Tel: +91 40 27204258; Fax: +91 40 27201672

obtain desired level of tenderization.

Materials and Methods

Sample preparation

Pectoralis major muscle from layer spent hen was collected post slaughter from a selected retail meat shop of Hyderabad. They were packed in Low Density Polyethylene (LDPE) bags and stored in refrigerator at 4±1°C for 24 hrs. After 24 hrs chilling, muscles were taken out of refrigerator and cut into small chunks and were randomly allotted for different treatments.

Ammonium hydroxide treatment

Different concentrations (0.1%, 0.5% and 1.0% v/v) of ammonium hydroxide (FCC) solution of pH 10.09, 10.46, and 10.6 respectively were prepared with distilled water and injected (single needle injector) and sprayed to each batch of meat chunks @ 15% v/w. For control batch only 15% v/w of distilled water was injected. After thorough mixing by hand, chunks were placed in polyethylene bags and kept at 4±1°C for 48 hours. After 48 hours of marination at 4±1°C, the raw meat chunks were evaluated for different quality parameters.

pH and water-holding capacity

The pH was determined by blending 10 g sample with 50 mL distilled water for 60 s in a homogenizer (Daihan Scientifics, WiseMix, HG-15D, Korea). The water-holding capacity (WHC) was determined by stirring 20 g of minced meat sample with 30 ml NaCl (0.6 M) followed by centrifugation at 5000 rpm for 15 min (Sorvall Biofuge Stratos, Thermo electron LED GmbH, D-37520, Osterode, Germany). The supernatant was measured and amount of water retained by samples was expressed in percentage (Wardlaw *et al*., 1973).

Per cent cooking yield

The weights of samples were recorded before (raw weight) and after cooking. Cooked weight was divided by raw weight and the result was multiplied by 100 to get per cent cooking yield.

$$\text{Cooking Yield (\%)} = (\text{Weight of cooked meat} / \text{Weight of raw meat}) * 100$$

Collagen content and solubility

Hydroxyproline (HP) content of the meat sample was determined based on the procedure of Nueman and Logan (1950) with few modifications as suggested by Naveena *et al.* (2004). Two-gram meat sample was hydrolysed with 40 mL of 6 160 N, HCl for 18 h at 108°C. The pH of the hydrolysate was adjusted to 7.0 and one mL of aliquot from this solution was used for hydroxyproline estimation. Absorbance was measured at 540 nm using UV-VIS spectrophotometer and the hydroxyproline content was determined by referring to a standard graph. Collagen content was calculated by multiplying hydroxyproline content with 7.14 and was expressed in mg/g tissue.

For collagen solubility 5 g of muscle tissue was cooked in water bath at boiling temperature for 30 min. The cooked meat was then homogenized with 50 mL distilled water at 4±1°C in a blender for 2 min. The extract was then centrifuged at 3000 g for 30 min. Aliquots of cooked out juice and centrifugate were hydrolyzed for 18 h at 108°C in hot air oven and soluble HP and collagen solubility were calculated as described below.

$$\text{\% HP Solubilized} = (\text{g HP in drip} + \text{g HP in cooked meat/g HP in raw meat}) \times 100$$

$$\text{\% Collagen solubility} = 7.14 \times \text{\% HP solubilised}$$

Protein extractability

Protein extractability was determined according to procedure of Joo *et al.* (1999). Sarcoplasmic proteins were extracted from 2 g sample using 20 ml of ice-cold 0.025 M potassium phosphate buffer (pH 7.2). The samples were homogenized and kept overnight at 4°C with frequent shaking. Samples were centrifuged at 5000 rpm for 20 minute and concentration of protein in the supernatant was determined by the Biuret method. For total protein (sarcoplasmic + myofibrillar) determination, same procedures for extraction, homogenization, shaking, centrifugation, and protein determination were used as described above, but with 40 ml of ice-cold 1.1 M potassium iodide in 0.1M phosphate buffer (pH7.2) as extracting solution. Myofibrillar protein extractability was obtained by difference between total and sarcoplasmic protein extractability.

Warner-Bratzler shear force value

Control and treated meat chunks were sealed in polyethylene bags and placed in water bath maintained at 100°C for 30 minutes, followed by overnight chilling at 4±1°C. Chilled samples were equilibrated to room temperature before texture measurement. After equilibration, the 1.25 cm cores were taken using tissue borer with muscle fibres parallel to the direction of the borer. The Warner-Bratzler shear force (WBSF) of the cores were measured using Texturometer (Tinius Olsen, Model

H1KF, 6 Perrywood Business park, Redhill, RH1 5DZ, England) with V-shaped stainless steel Blade (60° angle) and triangular whole in the middle. The cores were sheared perpendicular to the muscle fibre orientation with 75 Newton load range and a crosshead speed set at 200 mm/minute. The force required to shear the samples was recorded in Newton (N).

Muscle fibre diameter

Muscle fibre diameter was determined as described by Tuma *et al.* (1962). A small core (1.0 cm) of muscle tissue was fixed in 10% formal saline for 24 hours and then blended in a micro blender at low speed for 30 seconds. A drop of the homogenate was placed over a glass slide, covered with cover slip and observed under a microscope with 10x eyepiece containing a calibrated micrometer. The diameter of minimum of 10 fibres was measured and the average muscle fibre diameter was expressed in microns (μ).

Sodium Dodecyl Sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium Dodecyl Sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using the methods of Laemnli (1970) with electrophoretic apparatus (Tarsons, Model MC-01, Mumbai, India). The protein extracts obtained during protein extractability were used to run electrophoresis. About 10 μl of protein sample (containing 10 μg of protein) was used for loading the gel after denaturation by boiling for about 5 minutes. Electrophoresis was performed at a constant voltage mode of 72 V/slab at 30 mA for 5-6 h or until the tracking dye reached the lower end of the gel. The gel was removed and stained with coomassie blue for 4-5 h. The gels were then destained and photographed.

Statistical analysis

The overall experiment was replicated on three separate occasions. Statistical analysis was performed with the analysis of variance (ANOVA) using SPSS (SPSS version 13.0 for windows; SPSS, Chicago, IL, USA). Least square means for protected F-tests were separated by using Duncan's multiple range tests and were considered significant at $P < 0.01$ and $P < 0.05$.

Results and Discussion

The results of various Physico-chemical and histological qualities of spent hen meat chunks treated with different concentration of ammonium hydroxide (0%, 0.1%, 0.5%, and 1%) are shown in Table 1.

pH

In general, treatment with ammonium hydroxide (AHT) increased ($P < 0.01$) the pH in 1.0% and 0.5% samples compared to control and 0.1% AHT samples. This might be due to marination with ammonium hydroxide solutions having pH of more than 10.0. This is consistent with the findings of Nath *et al.* (2006) and Hamling and Calkins (2008) who reported that pH of meat increased as pump percentage of ammonium hydroxide is increased.

Table 1. Physico-chemical and histological qualities of Spent hen meat chunks treated with different concentration of ammonium hydroxide (0, 0.1%, 0.5%, and 1%)

Parameters	Control	AHT (0.1%)	AHT (0.5%)	AHT (1%)
Physico-chemical qualities[#]				
Meat pH**	5.66±0.04[a]	5.69±0.04[a]	5.81±0.01[b]	5.86±0.02[b]
Water-holding Capacity (%) **	22.5±1.44[a]	24.16±0.83[a]	27.67±0.17[b]	30.66±0.67[c]
Collagen content (mg/g tissue)	4.62±0.26	4.05±0.47	4.07±0.54	4.07±0.55
Collagen solubility (% total collagen) **	19.28±0.16[a]	19.81±0.78[a]	31.16±0.64[b]	49.41±0.45[c]
Protein Solubility[#] *(mg protein extracted/ 1 g total Muscle protein)*				
Sarcoplasmic protein solubility (mg/g)	89.33±0.67	92.00±1.15	89.67±3.38	93.50±3.50
Myofibrillar protein solubility (mg/g)*	122.00±2.08[a]	128.00±3.05[a b]	139.00±5.13[b]	121.50±3.50[a]
Total protein solubility (mg/g)*	211.33±0.67[a]	220.00±2.31[a b]	228.66±1.77[b]	215.00±7.00[a]
Shear force Analysis (N)[†]				
WBSF (N)**	48.99±1.69[b]	36.41±1.65[a]	34.23±1.62[a]	31.79±1.41[a]
Histological Parameters[†]				
Muscle fibre diameter (μ)*	37.56±0.18[b]	36.91±0.09[a]	36.37±0.16[a]	36.95±0.36[a]
Cooked Meat quality[#]				
Cooking yield (%)*	63.51±0.51[b]	57.88±1.45[a]	57.17±1.25[a]	59.06±0.59[a]

[#]Number of observations = 3
*significance level 0.05
[†]Number of observations = 21
**significance level 0.01
Means bearing same superscripts row-wise do not differ significantly

Water holding capacity (WHC)

Non-significant ($P > 0.01$) difference in WHC was observed between control and 0.1% AHT, whereas Significant ($P < 0.01$) increase in WHC was observed between 0.5% and 1% AHT samples. Similar results have been reported by Gupta et al. (1988) in ammonium hydroxide treated ground goat meat samples. Increase in pH results in large number of hydrophilic sites (Borton *et al.*, 1968) resulting in more binding of water molecules through hydrogen and ionic bonding to the hydrophilic sites of polypeptides. Water holding is caused by electrostatic repulsion between the myofibrillar proteins (myofilaments) which results in swelling of myofibrils or in some cases (with salt or high pH) even a partial solubilization of filaments due to repulsion between individual molecules (Hamm, 1977).

Collagen content and collagen solubility

The collagen content in spent hen breast meat ranged from 4.05 to 4.62 mg/g muscle tissue with non-significant ($P > 0.05$) difference between all groups. There was a significant ($P < 0.01$) and progressive increase in collagen solubility as the concentration of ammonium hydroxide treatment increased. The increased collagen solubility with use of ammonium hydroxide might be due to increased alkali soluble collagen on cooking. Similar observation was reported in beef by Irvin and Cover (1959). Similar

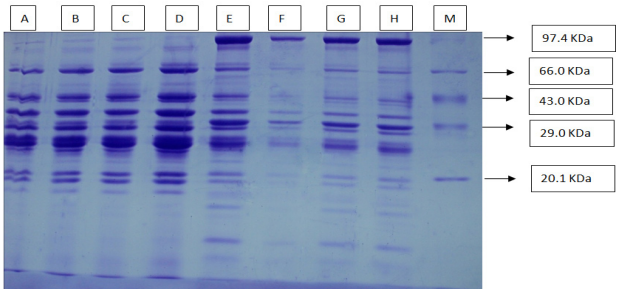


Figure 1. SDS PAGE photograph of spent hen meat after treating with various concentration of ammonium hydroxide

A- Control, B- 0.1 % AH, C- 0.5 % AH, D- 1 % AH: Water Soluble Proteins
E- Control, F- 0.1 % AH, G- 0.5 % AH, H- 1 % AH: Salt soluble proteins
M- Molecular weight marker
AH - Ammonium Hydroxide

increase in collagen solubility in buffalo meat chunks treated with ammonium hydroxide has been reported by Naveena *et al.* (2011b).

Protein solubility

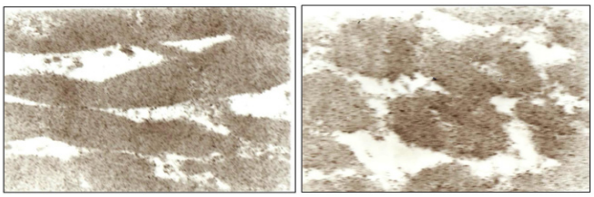
There was no significant ($P > 0.05$) difference in sarcoplasmic protein solubility among treatments. However, there was a significant ($P < 0.05$) increase in myofibrillar protein solubility of spent hen meat treated with 0.5% AHT and increasing the concentration to 1.0% AHT did not further increase the myofibrillar protein solubility. This increased solubility at 0.1% and 0.5% AHT followed by reduced solubility at 1.0% might be due to salting in and salting out mechanism of meat proteins (West *et al*., 1974). Increased protein solubility/extractability might be due to combined effect of higher pH, increased number of net negative charges and presence of salt (in this case ammonium hydroxide salt), Due to selective binding of ions, salt changes isoelectric point of meat proteins (Puolanne and Halonen, 2010).

Warner-Bratzler shear force (WBSF)

The WBSF for all AHT subjected samples were significantly ($P < 0.01$) lower than control. There was no significant difference in WBSF between different AH treated samples. The shear force values for control were significantly higher than ammonium hydroxide treated meat chunks. This might be due to increased collagen solubility and myofibrillar protein solubility with AHT. Reduction in shear force values with use of ammonium hydroxide was also reported by Cerruto-Noya *et al.* (2009) in beef and Naveena *et al.* (2011b) in buffalo meat.

Muscle fibre diameter

The muscle fibre diameter was found to be significantly ($P < 0.05$) higher in control compared to other samples. The reduction in muscle fibre diameter observed in current study might be due



A B

Figure 2. Transmission electron micrograph (longitudinal section) of raw spent hen *Pectoralis major* muscles a) control, b) ammonium hydroxide (AHT).

to solubility and/or disintegration of myofibrillar proteins (myofilaments) altering the orderly arranged structure of actomyosin with AHT. Tuma *et al.* (1962) suggested that, fibre diameter increases and tenderness decreases with increasing animal age. Hiner *et al.* (1953) also indicated that meat having small fibres was more tender than meat having large fibres.

Cooking yield

In the current study cooking yield decreased from 63.51% in control to 57.17 to 59.06% in AHT samples. Even though all treated samples had higher water holding capacity, the lower cooking yield in these samples compared to control indicate that the increased water might be mainly free water which is held by only capillary forces and will be lost during cooking. Reduction in cooking yield of beef samples treated with ammonium hydroxide was also reported by Hamling and Calkins (2008) and Nath *et al*. (2006).

Sodium-dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE)

The electrophoretic pattern (Figure 1) of sarcoplasmic proteins (Lane, A-D) shows no changes in band intensity or the numbers between control and AHT samples. The salt soluble proteins (Lane, E-H) revealed significant reduction in band intensity and disappearance of few bands especially in lane F (0.1% AHT) and G (0.5% AHT) indicating fragmentation and breakdown of myofibrillar proteins. These results strongly correlate with our protein extractability/ solubility findings. Previous researchers have also reported that, degradation of high molecular weight proteins (200-265 kDa) and increase in the number of low-molecular weight proteins (<25 kDa) is a result of proteolysis and degradation of myofibrillar proteins (Jorgova *et al*., 1989; Naveena *et al.*, 2011b).

Transmission electron microscopy

The transmission electron micrographs of spent hen muscles is shown in Figure 2. Eventhough, the photographs are not clear, orderly arranged structures

are visible in controls (A) compared to increased intermyofibrillar area in AHT samples (B). Similarly Taylor *et al.* (1995) reported that the degradation of intermyofibrillar linkages and costameres structures of muscle cells, and weakening of the thin filament/Z-disk have major roles in postmortem tenderization of muscle, this findings clearly indicates tenderization of spent hen meat with AHT.

Conclusion

The use of ammonium hydroxide lead to significant improvement of pH, WHC, myofibrillar and total protein solubility, collagen solubility and significantly reduced the WBSF. In many of the parameters studied 0.5 and 1.0% AHT significantly improved the tenderness compared to control and 0.1% AHT. However no difference was observed between 0.5% and 1.0% AHT. Hence it is concluded that 0.5% ammonium hydroxide would be sufficient to increase the tenderness and other qualities of spent hen meat when marinated for 48 hours at refrigeration temperature.

Acknowledgement

Authors wish to acknowledge the financial support of Indian Council of Agriculture Research, New Delhi. Authors also acknowledge the facilities provided by Director, National Research Centre on Meat, Hyderabad and support received from College of Veterinary Science, Hyderabad for this study.

References

Bilgili, S.F. 2002 Poultry meat processing and marketing - what does the future hold? Poultry International. September: 12-22.

Borton, R.J., Webb, N.W. and Bratzler, L.J. 1968. Emulsifying capacities and Emulsion stabilities of dilute meat slurries from various meat trimmings. Food Technology. 22: 506.

Cerruto-noya, C.A., Vanoverbeke, D.L. and Mireles Dewitt C.A. 2009. Evaluation of 0.1% Ammonium hydroxide to replace sodium tripolyphosphate in fresh meat injection brines. Journal of Food Science 74: C519-C525.

Dransfield, E., Casey, J.C., Boccard. R., Touraille, C., Buchter, L., Hood, D.E., Joseph, R.L., Schon, I., Casteels, M., Casentino, E. and Thimbergen, B. J. 1983. Comparison of chemical composition of meat determined at eight laboratories. Meat Science 8: 79.

Gupta, L.K., Garg, V. and Tiwari, R.P. 1988. Evaluation of ammonium hydroxide as preservative for ground meat. MIRCEN Journal 4: 431-437.

Hamling, A.E. and Calkins, C.R. 2008. Enhancement of beef chuck and loin muscles with ammonium hydroxide and salt. Journal of Animal Science 86: 967-971.

Hamling, A.E., Jenschke, B.E. and Calkins, C.R. 2008. Effect of aging on beef chuck and round muscles enhanced with ammonium hydroxide and salt. Journal of Animal Science 86: 1200-1204.

Hamm, R. 1977. Postmortem breakdown of ATP and glycogen in ground muscle: a review. Meat Science 1: 15.

Hiner, R.L., Hankins, O.G., Sloane, H.S., Fellers, C. R. and Anderson, E.E. 1953. Fibre diameter in relation to tenderness of beef muscle. Food Research 18: 364.

Irvin, L. and Cover, S. 1959. Effect of a dry method of cooking on the collagen content of two beef muscles. Food Technology 8: 655.

Joo, S.T., Kauffman, R.G., Kim, B.C. and Park, G.B. 1999. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water holding capacity in porcine longissimus muscle. Meat science 35: 276-278.

Keeton, J.T. 1983. Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. Journal of Food Science 48: 878-881.

Laemnli, U.K. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature 227: 680-685.

Mahendrakar, N.S., Dani N.P., Ramesh, B.S. and Amla, B.L. 1989. Studies on influence of age of sheep and post mortem carcass conditioning treatments on muscular collagen content and its thermoliability. Journal of Food Science and Technology 26: 102-105.

Nath, T.M., Hand, C.D., Everts, A.J., Everts, A.K.R., Wulf, D.M. and Maddock, R.J. 2006. Trained and consumer evaluations of five different beef muscles with or without pH enhancement using ammonium hydroxide. Downloaded from *http://www.meatscience.org/Pubs/rmcarchv/2006/presentations/RMC06_Abstracts.PDF.*

Naveena, B.M., Kiran, M. and Mendiratta, S.K. 2012. Post harvest technologies to deal with poultry meat toughness. World's Poultry Science Journal 69: 553-568.

Naveena, B.M., Muthukumar, M., Ramkrishna, C. and Kondaiah, N. 2011a. Effects of salt and ammonium hydroxide on the quality of ground buffalo meat. Meat Science 87: 315-320.

Naveena, B.M., Kiran, M., K.S. Reddy., C. Ramkrishna., S. Vaithiyanathan., Suresh, K.D. 2011b. Effect of ammonium hydroxide on ultrastructure and tenderness of buffalo meat. Meat Science. 88: 727–732.

Nueman, R.E and Logan, M.A. 1950. Determination of Hydroxyproline content. Journal of Biological Chemistry 184: 299.

Nurmahmudi and Sams, A.R. 1997. Tenderizing Spent Fowl Meat with Calcium Chloride III. Biochemical characteristics of tenderized breast meat. Poultry Science 76: 543-547.

Puolanne, E. and Halonen, M. 2010. Theoretical aspects of water-holding in meat. Meat Science 86:151-165.

Tuma, H.J., Venable, J.H., Wuthier, P.R. and Henrickson,

R.L. 1962. Relationship of fibre diameter to tenderness and meatiness as influenced by bovine age. Journal of Animal Science 21: 33-36.

Wardlaw, F.B., Maccaskill, L.H. and Acton, J.C. 1973. Effect of postmortem muscle changes in poultry meat loaf properties. Journal of Food science 38: 421-424.

West, E.W., Todd, W.R., Mason, H.S. and Bruggen, J.T.V. 1974. Proteins in WEST, E.W., TODD, W.R., MASON, H.S., BRUGGEN, J.T. (Eds). Textbook of Biochemistry. 4th edition pp. 261-355.