EXTRACTION AND CHARACTERIZATION OF WATER-SOLUBLE ELASTIN FROM SPENT HEN AND BROILER SKIN

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Abstract- Poultry by-products are not often processed into high-value products. Rather than being transformed into meal for animal feed, a large quantity of chicken skin could be used to produce elastin, which often being incorporated in the production of functional food or medicine due to its antioxidative properties. In this study, water-soluble elastin was successfully extracted from broiler and spent hen skin using four different solvents including NaCl, acetone, NaOH and oxalic acid (solubilizing liquid) prior to freeze-drying and Analyses including proximate and amino acid composition along with transmission electron microscopy (TEM) were carried out. It was apparent that the fat content of extracted elastin from broiler skin was notably higher ($P < 0.05$) than that of from spent hen's, with both samples recording less than 1% fat. Moreover, broiler skin elastin also had a higher protein content (68.3%) than spent hen's (67.8%). Both skin sources contained glycine as the major amino acid (20%), followed by glutamic acid, proline, alanine and arginine. The results of TEM indicated that the use of enzyme or further purification efforts should be incorporated along with the extraction methods used because of the presence of collagen and other debris in the resultant elastin.

Keywords: Elastin, Extraction, Poultry by-products, Broiler skin, Spent hen skin

I. INTRODUCTION

Elastin is a protein that is present together with collagen in connective tissue such as dermis, ligament, skin, tendon, or vascular wall .The functional form of the protein is that of a large, highly cross-linked polymer that organize as sheets or fibers in the extracellular matrix [5]. Theoretically, this protein is can be purified by exposing the tissues to high heat and extreme conditions of pH, resulting remaining residues of elastin due to its unique chemical composition and highly cross-linked nature. In the animal body tissue, the skin of poultry products and neck ligaments (ligamentum nuchae) of slaughtered livestock such as cattles and goats, contain a high concentration of elastin. Elastin is normally present in vivo as an insoluble protein having a three dimensional network structure. It is widely known that hydrolyzing such elastin with an acid or an alkali or treating it with an enzyme gives a wate-soluble elastin. Water-soluble elastin has the potential to be used in the field of regenerative medicine such as artificial blood vessels [1]. Thus, this preliminary study was carried out to extract water-soluble elastin from potential tissues of broiler and spent hen's skin and to compare both elastin's characteristics in terms of lipid, protein and amino acid composition.

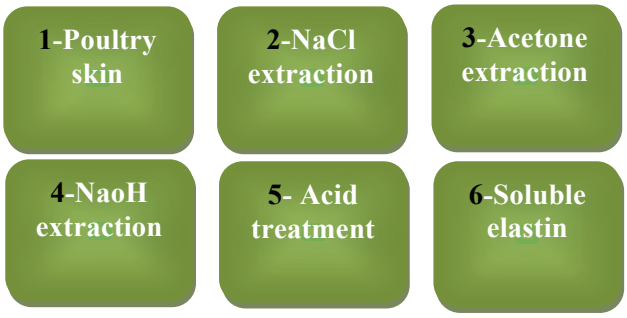
I. II. MATERIALS AND METHODS

Samples Extraction

Broiler's and spent hen's skin were cut into small pieces and homogenized in 1 M NaCl. After 24 h extraction with constant stirring in a cold room, the homogenate was centrifuged at 11000 rpm for 20 min for three times. Consequently, the pellet was washed with demineralised water, and was defatted with acetone for 1 hour for three times. The dry skin was then suspended in NaOH and heated for 15 min in a boiling water-bath with

constant shaking. After cooling and centrifugation, the residue was extracted again for 45min in NaOH at 100°C. The residual of NaOH-insoluble material was washed several times in water and lyophilized prior to further analyses. Subsequently, it will be solubilized at 100°C for 40 min to produce water- soluble elastin.

Fig 1. Major steps in elastin extraction [3]



Proximate Composition

The crude protein (Kjeldahl method) and fat content (Soxhlet extraction) of the raw materials and extracted elastins were estimated by the AOAC official method (AOAC 2005). The analyses were replicated three times.

Amino Acid Composition

Elastin samples were hydrolyzed in 6 mol/l HCl at 110 ºC for 16 h. The hydrolysate was dissolved in deionized water and filtered. The amino acid composition was obtained using a high performance liquid chromatography (HPLC), equipped with a Waters 410 Scanning Flourescence and AccQ Tag column (3.9 x 150 mm). AccQ Tag Eluent A and AccQ Tag Eluent B or 60% acetonitrile acid was used as the mobile phase (flow rate=1 ml/ min).

Transmission Electron Microscopy

The elastin powder was embedded in 1.5ml ethanol in tube to make a suspended solution. The tube was placed in ultrasonic bath for 10 min.

III. RESULTS AND DISCUSSION

The lipid and protein content as well as amino acid composition of the samples are as shown in Table 1 and Table 2, respectively. According to Table 1, broiler skin elastin had a significantly ($p < 0.05$) higher protein (68.25%) and lipid (0.95%) content than that of spent hen's. This could be contributed by the higher protein and lipid content of their raw materials.

Table 1: Lipid and protein content of elastin from broiler and spent hen skin

	Broiler skin	Spent hen skin
Lipid	$0.946^{a} \pm 0.066$	$0.656^{b} \pm 0.55$
Protein	$68.25^{a} \pm 0.052$	$67.83^{b} \pm 0.63$

The amino acid compositions of broiler and spent hen skin elastins are as summarized in Table 2. Both water- soluble elastins showed high proportion of glycine followed by proline, hydroxyproline and glutamic acid. As illustrated in Table 2, glycine was the most dominant amino acid in both source of elastins, by which broiler skin elastin showed a higher content of glycine (20.89 %) than that of spent hen's (19.75 %). This result also exhibited that the Lansing method could not remove hydroxyprolin from the extracted elastins. Hydroxylysine, which is characteristic for collagen, and absent in pure elastin, is the main amino acid in collagen [1]. Moreover, the presence of methionine at low concentration in elastin from broiler and spent hen skin suggested that this extraction method can be further improved by highly possibly involving enzyme to eliminate all methionine from final elastin products. Approximately 1.3% to 4.2% of the constituent amino acids of the water-soluble elastin were comprised of lysine, histidine and arginine.

Table 2: Amino acid composition of elastin from broiler and spent hen skin

Amino acids	Broiler skin	Spent hen skin
Hydroxyl prolin	$9.88^{a} \pm 0.62$	$10.77^{b} \pm 0.22$
Aspartic acid	$7.71^{b} \pm 0.08$	$7.56^{a} \pm 0.43$
Serine	$1.65^{a} \pm 0.02$	$2.32^{a} \pm 0.02$
Glutamic acid	$12.38^{b} \pm 0.38$	$11.63^{a} \pm 0.06$
Glycine	$20.89^{a} \pm 0.12$	$19.75^{b} \pm 0.39$
Histidine	$1.27^{a} \pm 0.1$	$1.23^{a} \pm 0.08$
Arginine	$4.02^{a} \pm 0.04$	$5.28^{a} \pm 0.12$
Threonine	$0.64^{a} \pm 0.04$	$0.86^{a} \pm 0.03$
Alanine	$8.74^{a} \pm 0.14$	$8.48^{a} \pm 0.18$
Proline	$11.54^{a} \pm 0.08$	$11.39^{a} \pm .05$
Tyrosine	$1.56^{a} \pm 0.04$	$1.55^{a} \pm 0.02$
Valine	$3.16^{a} \pm 0.04$	$2.95^{a} \pm 0.02$
Methionine	$1.84^{a} \pm 0.03$	$1.80^{a} \pm 0.07$
Lysine	$4.52^{a} \pm 0.03$	$4.22^{a} \pm 0.04$
Isoleucine	$4.62^{b} \pm 0.04$	$2.30^{a} \pm 0 .02$
Leucine	$4.62^{a} \pm 0.04$	$4.33^{a} \pm 0.05$
Phenyl alanine	$3.46^{b} \pm 0.8$	$2.81^{a} \pm 0.05$
Cysteine	$0.1^{a} \pm 0.01$	$0.12^{a} \pm 0.03$
Tryptophan	$0.19^{a} \pm 0.05$	$0.18^{a} \pm 0.08$

Note: $^{a-b}$ means with different letters in rows are significantly different ($P < 0.05$)

Electron Microscopy

TEM was used to identify the presence of impurities (e.g. collagen and microfibrils) in the elastin preparations. Contaminations are visible as electron dense material on the electron translucent elastin. Our TEM results proven that Lansing method were not able to completely removed impurities as evident by the presence of non-elastinous material of microfibrillar nature.

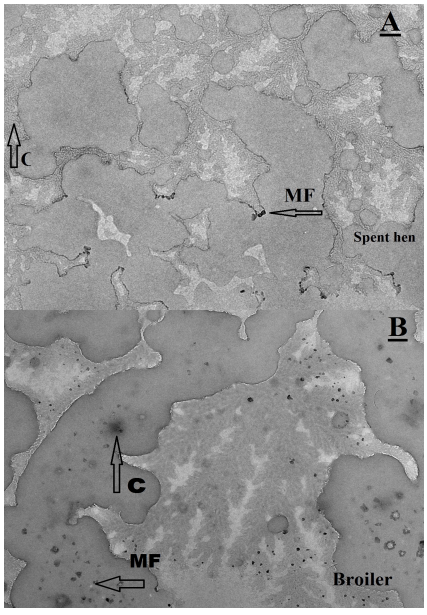


Fig 2: Transmission electron micrographs of water-soluble elastin from (A) spent hen and (B) broiler. Note: **C** (collagen), **MF** (microfibrillar)

IV. CONCLUSION

This study outlined the solvent extraction method to produce water-soluble elastin from broiler and spent hen skin. It was evident that both resultant elastins were of high protein and low lipid content. This study also suggested that an improved method to produce pure elastin, preferably involving enzyme, may aid in eliminating the presence of hydroxyprolin and methionine, which are the inherent properties of collagen protein.

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REFERENCES

1. Daamen, W.F., Hafmans, T., Veerkamp, J.H., Kuppevelt, T.H. 2001. Comparison of five procedure for the purification of insoluble elastin. Journal of Biomaterials. 22: 1997-2005
2. Jacob, M.P., Horenebeck, W. 1985. Isolation and characterization of insoluble and kappa-elastins, In: Robert L, Moczar M, Moczar E, editors. Frontiers of matrix biology, Vol.10.Basel:Karger. page. 92-133.
3. Lansing, A.I., Rosenthal, T.B., Alex, M., Dempsey, E.W. 1952. The structure and chemical characterization of elastic as revealed by elastase and by electron microscopy. The Anatomical Record. 114: 555-575.
4. Soskel NT, Sandburg LB. A comparison of six methods of extracting elastin residue from hamster lungs. Exp Lung Res 1983;4:10919.
5. Starcher, B.C., Galione, M.J. 1976. Purification and comparison of elastin from different animal species. The Analytical Biochemistry. 74: 441-447.