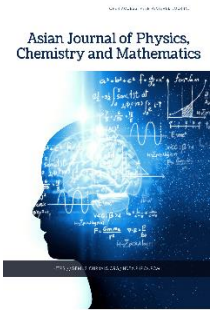

Extracting Of Hyaluronic Acid from Different Sources

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ABSTRACT

Hyaluronic acid (HA) is the most common polymer existing in different tissues and liquids of vertebrae animals as well as humans. Hyaluronic acid is known to belong to glycosaminoglycans class. Mucopolysaccharides were isolated from mucus, to which they give viscous lubricating properties. These properties, in turn, are related to glycosaminoglycan's ability to bind to a significant amount of water.

Recently, there has drawn increased attention to extract HA from the tissue of vertebrate animals or from the bacteria that creates the protecting capsule from the polysaccharides. But until now HA extracted mainly from rooster comb. So the main object of this research work is to extract HA from eggshells and bovine tissue; to review obtained substances through the uses of IR analysis.

Keywords: Hyaluronic acid, extraction.

Hyaluronic acid is a natural polysaccharide glycosaminoglycan of the type (-GlcNAc-GIcUA-)$_n$ where GlcNAc is N-Acetyl-D-glucosamine and GlcUA is D-glucuronic acid. This polymer is a constituent of various connective tissues and is always found associated with protein. In the vitreous body, hyaluronic acid is found together with collagen, serum proteins, and non-plasma proteins. HA is responsible for playing a part in several vital roles in the body such as cell differentiation, tissue hydration and lubrication, and nutrient diffusion.[1] The most important functions of HA in the body which performing the role of joint lubrication that supports regeneration, hydration and elasticity of fabrics are determined by its structural features and properties. Such properties are polyelectrolyte nature, high water-holding capacity, solubility in water, high viscosity and ability to gelation.[2] The human body weighing 70 kg contains 15 g of HA. It can also be found in the places where friction occurs: the joints, tendons, sheaths, pleura, and the pericardium. As it is mentioned above HA retain many water. Because of this, it helps the skin look youthful and healthy. When skin loses hyaluronic acid, it also loses its ability to retain moisture. The viscoelastic nature of hyaluronic acid as well as its biocompatibility

and non-immunogenicity has rendered it to be used in a number of medical applications such as supplementation of joint fluid in arthritis and acceleration of tissue repair and wound healing[3]. Cross-linking HA with copolymers provides the ability for the formation of hydrogel or nanogel networks[4]. Depending on HA concentration and molecular weight, this biopolymer determines the physiological functions of the cells, tissues and organs, in functions of water-binding, ionic exchange, molecule's size-dependent diffusion and impermeability towards large molecules and cells. As a consequence, it provides protection from penetration of high molecular weight toxins and microbiological invasions. Hyaluronic acid helps wounds heal faster by regulating inflammation levels and signaling the body to build more blood vessels in the damaged area. Because its concentrations increase when there is damage in need of repair[4]. Beside this, there are also many number of biomedical application and function of HA. For example, it is used in eye surgery, corneal transplant, used to osteoarthritis, preserve bone strength, relieve joint pain by keeping bones well lubricated and etc.

The last few decades witnessed a growing number of publications on role of hyaluronan in fertilization (in direct correlation with the development of numerous programs of in vitro fertilization), cell division and migration, angiogenesis, wound healing and tissue regeneration. It is known today that HA is actively involved in the regulation of cell division, migration, differentiation and tissue and organ regeneration at all stages of organism development or ontogenesis.[5]

Hyaluronan is involved in many biological processes and is present in almost all body tissues of vertebrates where it plays a role in the regulation of cellular activities. It speeds up (or slows) cell division, influences migration, and is involved in the reorganization of chromatin structure and gene switching. Hyaluronan is localized in the nucleus, the cytoplasm and the intercellular matrix, where it interacts with receptors on the cell surface and intercellular matrix proteins.[6] HA is involved in the process of a cell's adaptation to physical and chemical exposure, the process of fertilization, embryogenesis, angiogenesis, inflammation, regeneration and tumor growth.[3] It can exhibit additive, synergistic and antagonistic properties with related sulfated polysaccharides and between hyaluronan oligosaccharides with different molecular weights. Hyaluronic acid and its salts, has been extracted from sources such as rooster comb (1), vitreous humor (2) and umbilical cords. It is also known to exist in group A and C streptococci, which are ideally suited for the biosynthesis of HA due to the abundant availability of HA and since in this organism HA is the only polymer into which glucuronic acid is incorporated.[7]

2. Experimental Procedures

2.1 The extraction of HA from bovine tissue

The extraction of HA from bovine tissue includes the following stages. The grounded animal tissue was treated with 95% ethanol, denaturized with chloroform for 24 h. Treatment was repeated several times until the solution stayed colorless and transparent. Then HA was extracted with mixture of water and chloroform 20:1. The mixture was stirred and let to stay without stirring for 24 h at 4–25°C, the mixture filtered, and the extraction repeated twice. Aqueous sodium chloride and chloroform 1:1 were added to combined extracts and a mixture was stirred for 3–5 h at 4–25°C. Then the mixture was kept until full fractions separated and organic fraction was isolated. The aqueous fraction was treated with hydrochloric acid till pH 4–5 and the equal volume of chloroform was added again. The procedure was repeated until the chloroform layer became transparent. The pH value of hyaluronan solution was adjusted to 4.0-5.0 by adding HCl. Finally, ethanol was added to the solution for 3:1 ratio, and white sediment of the HA was formed at the bottom of flask.

2.2 Extraction of HA from bovine tissue using sodium citrate

First, the bovine tissue is washed with cold water. Then it is dried and 10 g bovine tissue was ground in an electric grinder (yielded pieces in about 0.5 cm in size), then placed in 30 ml of acetone in a refrigerator to remove fat from the bovine tissue. This was repeated two

times. After the last extraction and draining, the remaining acetone was evaporated in a stream of air. The defatted and dried bovine tissue was extracted two times with 5% of sodium citrate at a ratio of 1:6 and extracted for 24 h. Each time the viscous fluid was collected by squeezing through a cotton cloth. After the extraction, the bovine tissue residue was discarded. The extracts are then combined and the polysaccharide is precipitated with two volume of methanol and 1 ml 10% of HCl. Then the precipitate was desiccated by freeze-drying.

2.3 Extraction of HA from eggshell using saline solution

First, eggshells were removed from protein membrane and carefully grounded in an IKA-WERKE M20 electric grinder. 0,9% saline solution was added to the grounded eggshell at a ratio of 3:1 respectively and brought to a boil at 40-45ºC, after which the extract was separated. The extraction process is repeated two times and the eggshell was discarded. The extracts were then combined and 4 volume of ethanol was added to the aqueous extracts. The precipitate was centrifuged. The final product was dried by lyophilization.

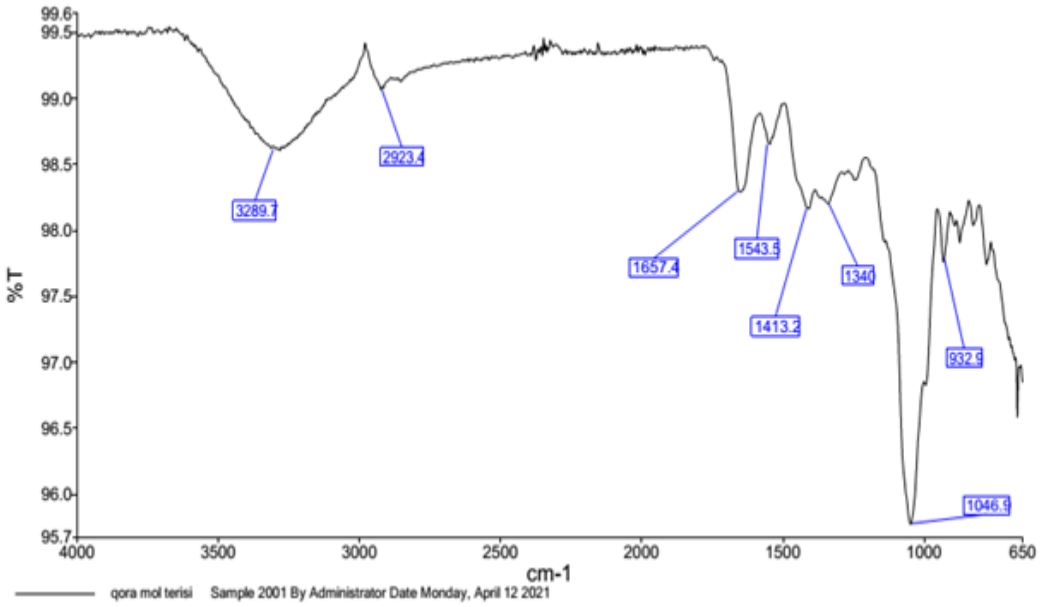
2.4 Extraction of HA from eggshell using sodium citrate

Production of HA includes two-stage extraction of the homogenized eggshell with 5% sodium citrate at a ratio of 1:10. The combined solutions were treated with 2 volume of methanol and 1 ml 10% of HCl. Then the mixture was stirred for 10 min to obtain precipitate. The final product was dried by lyophilization.

3. Results and Discussion

We used BRUKER Fourier - Spectrometer (4000-450 cm^{-1}), the total reflection FTIR analyses were conducted on to characterize the functional groups and bonds of the HA from different sources and compared with Infrared spectra (FT-IR) of Standard Hyaluronic Acid FTIR. The IR spectrum of HA from bovine tissue and eggshell are listed below (figure-1 and figure – 2):

a)



b)

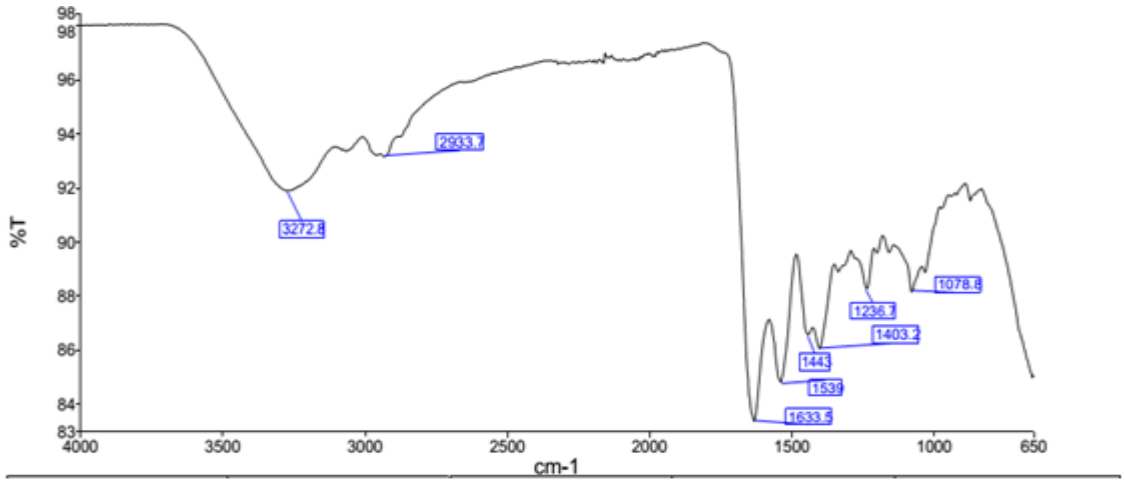
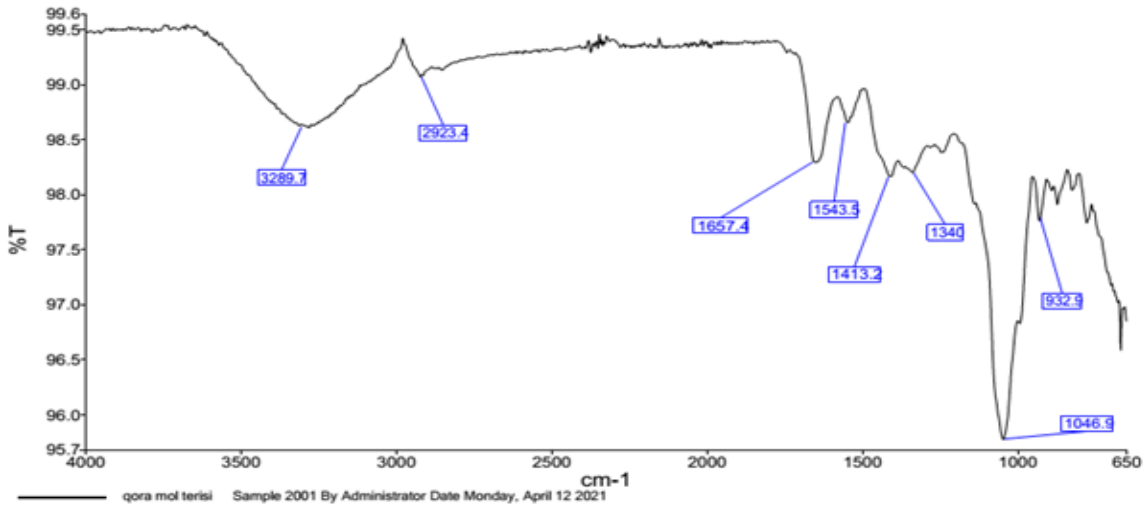


Figure-1 IR spectrums of bovine tissue using saline (a) and citrate (b) solution

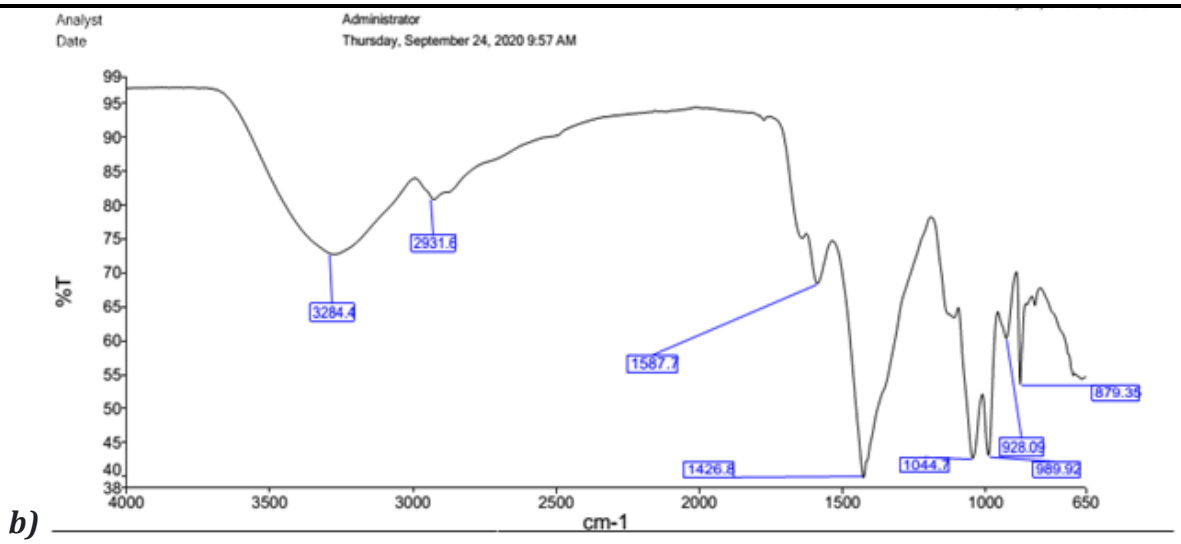
The following results (table-1) were obtained during IR spectrums of bovine tissue

Table-1 Results of IR spectrums of bovine tissue and its comparison with standard HA

№	IR bands of standard HA	IR bands of bovine tissue extracting by using citrate solution	IR bands of bovine tissue extracting by using saline solution	Functional groups
1	3349 cm^{-1}	3289.7 cm^{-1}	3272.8 cm^{-1}	-OH
2	2925 cm^{-1}	2923.4 cm	2933.7 cm^{-1}	-C-H
3	1638 cm^{-1}	1543.5 cm^{-1}	1539 cm^{-1}	amid II
4	1409 cm^{-1}	1413.2 cm^{-1}	1403.2 cm^{-1}	-N-H
5	1023 cm^{-1}	1046.5 cm^{-1}	1078.8 cm^{-1}	-C-O-C



a)



b)

Figure -2. IR spectrums of eggshell using saline (a) and citrate (b) solution

Table-2. Results of IR spectrum of eggshells and its comparison with standard HA

№	IR bands of standard HA	IR bands of eggshells extracting by using citrate solution	IR bands of eggshells extracting by using saline solution	Functional groups
1	3349 cm^{-1}	3284.4 cm^{-1}	3289.7 cm^{-1}	-OH
2	2925 cm^{-1}	2931.6 cm^{-1}	2923.4 cm^{-1}	-C-H
3	1638 cm^{-1}	1587.7 cm^{-1}	1657.4 cm^{-1}	Amid II
4	1409 cm^{-1}	1426.8 cm^{-1}	1413.2 cm^{-1}	-N-H
5	1023 cm^{-1}	1044.7 cm^{-1}	1046.9 cm^{-1}	C-O-C

Through analyses of the FT-IR, it was possible to confirm the structure of Hyaluronic Acid from animal tissue when comparing with the Standard Hyaluronic Acid, through the presence of bands found in all spectra of FT-IR.

References

1. J.P. Chen. Functionalized temperature-sensitive copolymer for tissue engineering of articular cartilage and meniscus, Colloids and Surfaces A. 313-314 (2008) 254-259.
2. H. Tan, et al., Thermoresponsive injectable hyaluronic acid hydrogel for adipose tissue engineering, Biomaterials 30 (36) (2009) 6844-6853.
3. Park, S.N.; Lee, H.J.; Lee, K.H. & Suh, H., Biological characterization of EDC cross-linked collagen-hyaluronic acid matrix in dermal tissue restoration, Biomaterials, Vol.24, (2003) 1631-1641.
4. Balazs, E.A. (1967) Ber. Dtsch. Ophthalm. Ges. 68, 536—572
5. By V. N. Khabarov, P. Y. Boykov, M. A. Selyanin. Hyaluronic Acid: Production, Properties, Application in Biology and Medicine.
6. Spicer, A.P., McDonald, J.A. (1988) Characterization and molecular evolution of a vertebrate hyaluronan synthase gene family, Journal of Biological Chemistry.
7. Tai-Jin Kim, Yoon-Jun Lee, and Duk-Jung Kim. Separation of Hyaluronic Acid from Plant and Animal Tissues.