

ARTICLE

WASTE TO WEALTH: ALTERNATIVE SOURCE OF GLYCOSAMINOGLYCANS (GAGS) FROM SEA FOOD WASTE

Nurul Haida Idrus, Nina Suhaity Azmi*, Jaya Vejayan Palliah

Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Kuantan, Pahang Darul Makmur, MALAYSIA

ABSTRACT

Glycosaminoglycans (GAGs) are compounds that were used to treat various diseases. Generally GAGs widely used in biological and medical approaches. Osteoarthritis (OA) is the one example of disease that used GAGs by enhancing the production of cartilage matrix components in the joints. In Malaysia, management of organic waste such as fish bone is the focus point to achieve environmental sustainability. Sea food waste from *Lates calcarifer* was used as the model to extract GAGs where a few tests were carried out to verify the presence of GAGs. These fish is popularly sought-after and this will provide its unwanted parts (gills and viscera) into therapeutically and economically viable solution for society. Then, the invention convert this waste to wealth which help for the better utilization of seafood waste. Analytical testing were carried out to determine the similarity of the functional group that were presence in sample with standard provided. Instead of that, Blyscan assay kit was used to determine the presence of GAGs in crude sample. From the result obtained, GAGs are well distributed in sea food waste of *Lates Calcarifer*. Instead of that, waste management can be minimize by convert waste to wealth product

INTRODUCTION

Waste management is gradually becoming a serious concern globally. One of the approaches to reduce waste management is by using reduce, recycle, and reuse (3R) methodology their practices are at the low to moderate levels. There are lack of policy and participation from the public. In April 2009, the Ministry of Energy, Green Technology and Water was established to manage green technology development in Malaysia. Private sectors were encouraged to invest in green technology by promoting the usage of more environmentally sound waste management towards dealing the changes in the global environment in order to minimize the waste management [1]. According to the Department of Statistic, Malaysians among the world's top fish consumer and spend about RM100 a month for variety of fish. There are 54% of consumers that were reported eat fish once to three times a week [2]. Toward sustainable waste management, waste to wealth should be implemented among the consumer in order to minimize the global warming phenomenon from sea food waste. The commercial fish processing industry creates a problem when only a flesh are used to make a product and a huge number of unwanted parts will be dumped as waste. Waste from fish processing have active ingredients that can produce high value product. Currently, collagens are the example of the product that can be extracted from seafood waste and widely used in pharmaceutical field [3]. Environmental problems might occurs without proper utilization of waste. The fish waste was reported good source of protein content and polyunsaturated lipid [4]. Potential marketable shows active ingredients that present in food waste can create natural product such as proteins, polysaccharides, fibers, flavor compounds, and phytochemicals [5].

Glycosaminoglycans (GAGs) are large complex polysaccharide chain consists of repeating disaccharides unit. It composed of repeating disaccharide unit of hexosamine which are glucosamine or galactosamine and uronic acid which are glucuronic acid or iduronic acid. GAGs have the ability to maintain negative charge polymer towards cell membrane and also as hydration sites which maintain the properties of mucous membrane. GAGs commonly found in mammalian species and can be classified based on their interaction with the enzyme. There are six classifications of GAGs which are chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, heparin and hyaluronic acid [6-8]. GAGs can be extracted from cartilage which but too expensive for commercialization purposes. Even though cartilage has low value by product but it is rich in chondroitin sulfate which is one of GAGs members [9]. It has been reported that, cartilage contains approximately, 10% by dry weight a proteoglycan (PG) that was known as aggrecan where different types of GAG chains are covalently attached. In 1884, chondroitin sulfate (CS) which is one types of GAG that was first isolated and also been reported to treat osteoarthritis. On the other hand, from the previous study, CS is extracted from various natural sources such as, shark, chicken and also bovine cartilage [10].

MATERIALS AND METHODS

Extraction of GAGs

Sea bass waste of *Lates calcarifer* was used as raw material in this research to extract GAGs. The sample was obtained from seafood waste restaurant in Kuantan, Pahang. The sample was weighed and separated into their parts which are, gills, viscera and gall bladders. Then, the sample was washed with sodium chloride (NaCl) to remove blood and other impurities. Instead of that, NaCl was used to prevent any growth of microorganism on sample. The procedure then was continued with removal of protein where the sample was soaked with ethanol for 14 days. The purpose of this method is to remove fat in the sample. During this

KEY WORDS

Waste Management,
Glycosaminoglycans
(GAGs), Sea Food Waste

Received: 15 Oct 2016
Accepted: 14 Nov 2016
Published: 4 Dec 2016

*Corresponding author
Email: nina@ump.edu.my

process, ethanol should be replaced three time for 14 days. Urea was used to denature protein and ribonucleic acid (RNA) in the sample and also making the intermolecular ones stronger. After that, the sample was kept at room temperature with soft agitation for overnight with the mix of digestion buffer. Precipitated protein was removed by centrifugation. Supernatant from the sample was collected and treated with 3 volume of ethanol while the residue was treated with of NaOH. To ensure the presence of precipitate in the sample, keep it in the chiller for overnight. On the next day, the second centrifuge was carried out to collect the residue which is the pellet that contain GAGs. When the method was commenced, the residue was collected and treated with 0.1 M NaOH. The crude then was kept in the freezer at temperature -80°C for further lyophilization process.

Characterization of GAGs

GAGs can be characterized using Blyscan Sulfated Glycosaminoglycans Assay that was, purchased from a British company Biocolors. This assay is a quantitative dye-binding method for the analysis of sulfated proteoglycans and glycosaminoglycan. Besides that, this assay can be used to determine the O- and N-sulfated glycosaminoglycan ratio within the sample. The standard plots were obtained using chondroitin sulfate standard solution and values of test samples were determined by using linear equation of standard graph [11-13]. Instead of using blyscan kit, Bradford Protein Assay was used to determine the protein solution that was presence in the sample solution. This assay relies on the binding of the dye Coomassie Blue G250 to protein. It widely used because of it simpler, faster and most important is most sensitive [14-16].

RESULTS AND DISCUSSIONS

Determination of protein content in crude sample

The Bradford Protein Assay was used to measure the concentration of protein in sample. The principal of this assay based on binding of protein molecule to Coomassie dye under acidic conditions. Bovine serum albumin (BSA) was used as the standard to measure the protein in sample [17, 18]. Different concentration of GAGs were prepared and dilute with 1mL of distilled water. This stock solution then was diluted with 1mL of Bradford reagent that was prepared before carried out this test. Different concentration of BSA was used to prepare the standard calibration curve starting from weight 0 µg to 10 µg at concentration 2 mg/mL. The sample and standard then were incubated for 20 minutes before proceeded with absorbance measurement at 595 nm using UV-visible spectrophotometer. The selected wavelength (595 nm) is the maximum peak where the relationship between protein and absorbance was occurred. The result showed that, concentration of protein sample that was measured from the standard calibration curve was 0.2056 mg/mL which is lowest protein in 100 µl of GAG sample. The protein concentration was considered low because during extraction process, 70 % of ethanol was used to remove protein. Instead of that, UV scanning also being carried out after extraction process to detect the presence of protein at wavelength 280 nm which is 0.0733 A. Animals tissue have the higher protein yield content. Thus, various protein solubilization are used to lower the protein content in the sample [19]. Interference of protein content in crude sample may affect the process of recovery the GAGs compound from sea food waste.

GAGs determination in sea food waste

Blyscan assay is the method that was used for the analysis of sulfated proteoglycans and glycosaminoglycans. The sensitivity of this quantitative dye-binding is 0.5 µg and it will take about one hour to run the assay. The absorbance peak for Blyscan dye in the dissociation reagent is 656nm. This absorbance is suitable to use with most colorimeters and microplate readers with a red filter. Total standard of sulfated GAGs using Blyscan assay kit was plotted. The statistical measure of data which is R2 is 0.9969 and it is the best coefficient of determination. Total GAGs that was presence in aliquot sample by using this assay is 1.9053 µg/ml. Equation 1 was used to determine the total GAGs that was presence in the crude sample.

$$y = 0.2122x \quad (1)$$

Table 1: Extraction yield of sample using 1151.59 g of raw material will produce 17.2 % of crude GAGs

Sample (g)	Before Freeze Dry (g)	After Freeze Dry (g)	% Yield
1151.59	198	7.67	17.2

Instead of using internal part of fish, shrimp head always used to extract GAG because it contains source of digestive enzyme. Recent study has demonstrated that, 79 mg of sulfated glycosaminoglycan can be obtained from 0.236 kg of shrimp head waste as well as some properties and technological applications [20]. In this research study, about 1151.59 g of raw material from the sea bass waste was used and produce 7.67 of GAGs. [Table 1] shows weight of the sample during the experimental process. According to the marine prospective, shark fins have commonly used source of CS which is the most common types of GAG. Since this source of GAG highly demanded, another source of GAG has been reported [21, 22].

Analytical analysis of GAGs

The most common method that usually used in the identification test is using Fourier Transform Infrared Spectroscopy (FT-IR). [Fig. 1] shows the result for of GAGs in the Lates calcarifer and it was compared with the chondroitin sulfate standard. The peaks were compared with the standard to find the similarity of the sample according to the provided standard. The absorbance of the maximum peak of the sample at 3456.54 cm⁻¹ while compare to the standard is 3344.87 cm⁻¹. Throughout this research study, there are several functional groups that were determine that have similarity with standard provided. Identification test FTIR showed that the peak at 3456.54, 1667.23, and 1627.70 cm⁻¹ in FTIR spectrum shown strongly suggesting the chemical entity as chondroitin 4 sulfate (CS). The absorption was read at 400 to 4000 cm⁻¹ as shown in [Fig. 1, Table-2]. The band at 1667.23, and 1627.70 cm⁻¹ were detected due to the stretching or deformation vibration of C-O-H bands and this bands were suggesting the presence of combined carboxylate with amine and sulphate [23, 24]. Further structural characterization of GAGs are required in order to obtain the confirmation between different samples and standard.

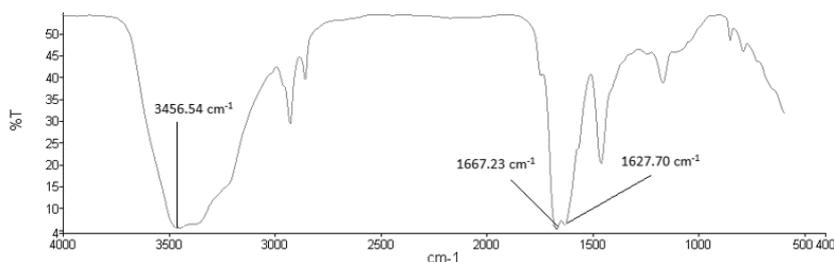


Fig. 1: Characterization of GAGs using FT-IR.

Table 2: Functional groups and frequencies (peak) that were presence in crude sample of GAGs. The absorbance were measured between wavelength 4000-400 cm⁻¹. Each sample used the Potassium Bromide (KBr) ratio method in sample preparation to form disc.

Functional Group	Peaks in Sample (cm ⁻¹)
N-H bends	3456.45 and 1627.70
C= O stretch	1667.23

Instead of using FT-IR for characterization, ¹H NMR also used to determine the structure of molecule presence in sample including large protein [24]. Nuclear magnetic resonance (NMR) is a spectroscopy technique that based on the absorption of electromagnetic radiation in the radio frequency 4 to 900 MHz by nuclei of the atoms. It has become an indispensable analytical technique in medicine, biology, chemistry, physics and food science. This instrument connect with the sample by electromagnetic wave in the radiofrequency range. According to the previous study, sulfation position at 4-sulfated of GAGs is between range 4.47-4.78ppm. Meanwhile, sulfation position for 6 sulfated at range 4.19-4.21ppm. Regarding the information from the graph, the structure and functions of GAGs can be identified from the graph. Classification of GAGs can be identified according to the identities of the hexoamines unit, hexorunic acid and the sulfation of both residue. Hence, it can be conclude that, sea bass waste has the presence of GAGs AS shown in [Fig. 2] [25, 26].

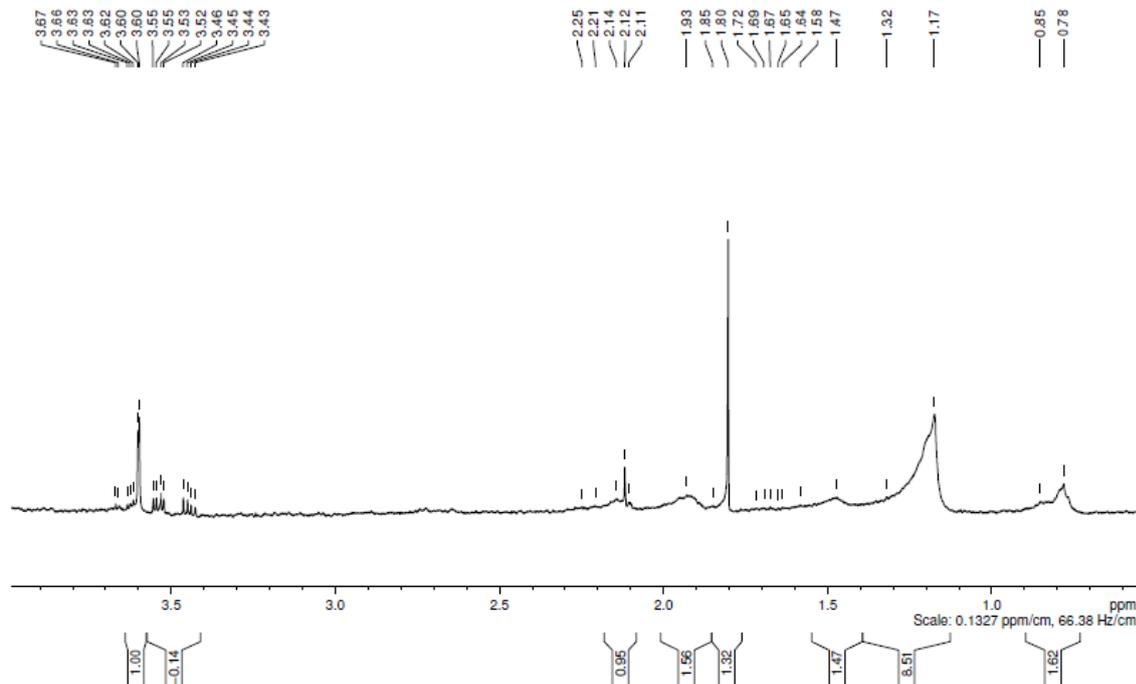


Fig. 2: Result of ¹H NMR of GAG at room temperature.

Glycosaminoglycans (GAGs) have been successfully extracted from Lates Calcarifer. The crude extract has been characterized using FTIR and NMR analysis to quantify the presence of GAGs. Besides that, the total amount of sulfated GAGs in the sample can be measured by Blyscan assay. It shows that 1.9053 µg/ml of GAGs concentration in an aliquot of 50 µl sample solution. Waste also useful to produce beneficial product and can solve the environmental problems.

CONFLICT OF INTEREST

There is no conflict of interest.

ACKNOWLEDGEMENTS

The authors thank to the UMP GRS scheme for postgraduate research students, and we are grateful for the assistance of technical staff in Faculty of Industrial Sciences & Technology, University Malaysia Pahang. The authors thank others researcher that involve in this research.

FINANCIAL DISCLOSURE

This paper is based on research supported by University Malaysia Pahang under operation research grant (UMP RDU130308), Ministry of Higher Education for Research Acculturation Grant Scheme (RAGS RDU 131406) and Fundamental Research Grant (FRGS RDU 140131).

REFERENCES

[1] Sreenivasan J, Govindan M, Chinnasami M, Kadiresu I. [2012] Solid waste management in Malaysia: a move towards sustainability, Waste management: an integrated vision. InTech p. 348.

[2] Aruna P. [2014] Malaysian eats more fish than Japanese, reveals study, in The Star, ed.

[3] Raghuraman H. [2013] Extraction of Sulfated Glycosaminoglycans From Mackerel And Herring Fish Waste.

[4] Nurdiana H, Siti Mazlina M, Siti nor Fadhilah M. [2008] Optimization of protein extraction from freeze dried fish waste using response surface methodology (RSM), International Journal of Engineering and Technology. 5:48-56.

[5] Baiano A. [2014] Recovery of biomolecules from food wastes—A review, Molecules. 19:14821-14842.

[6] Knoll W, Azmi NS, Fernig DG. [2013] Heparan Sulfate Surfaces to Probe the Functions of the Master Regulator of the Extracellular Space, in Handbook of Biofunctional Surfaces, ed: Pan Stanford Publishing. 591-615.

[7] Marina MS, Nina Suhaity A, Hasbi M, Rahim A. [2014] Review: Glycosaminoglycans (GAGs) Versus Cancer, Journal of Environmental Bioremediation And Toxicology. 2:58-61.

[8] Lokwani R. [2015] Beyond Anticoagulant: Heparin as a Potential Anti-cancer Agent, Journal of Biochemistry, Microbiology and Biotechnology (e-ISSN 2289-5779). 2:76-82.

[9] Nakano T, Ikawa N, Ozimek L. [2000] An economical method to extract chondroitin sulphate-peptide from bovine nasal cartilage, Canadian agricultural engineering. 42:205-208.

[10] Davies N, Roubin R, Whitelock J. [2007] Characterization and purification of glycosaminoglycans from crude biological samples, Journal of agricultural and food chemistry. 56:343-348.

[11] Ma BI, Zhou PH, Xie T, Shi L, Qiu B, Wang Q. [2015] Inhibition of interleukin-1beta-stimulated dedifferentiation of chondrocytes via controlled release of CrmA from

- hyaluronic acid-chitosan microspheres, BMC musculoskeletal disorders. 16(1).
- [12] Pievani P, Azario I, Antolini L, Shimada T, Patel P, Remoli C, et al. [2015] Neonatal bone marrow transplantation prevents bone pathology in a mouse model of mucopolysaccharidosis type I, *Blood*. 125:1662-1671.
- [13] Sutherland AJ, Beck EC, Dennis SC, Converse GL, Hopkins RA, et al. [2015] Decellularized cartilage may be a chondroinductive material for osteochondral tissue engineering. *PLoS one*. 10:0121-0966.
- [14] Kruger NJ. [1994] The Bradford method for protein quantitation, *Basic protein and peptide protocols*. 9-15.
- [15] Ernst O, Zor T. [2010] Linearization of the Bradford protein assay, *JoVE (Journal of Visualized Experiments)*. 1918-1918.
- [16] Walker JM. [1996] *the protein protocols handbook*: Springer Science & Business Media.
- [17] Bradford MM. [1976] A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analytical biochemistry*. 72:248-254.
- [18] Noble JE, Bailey MJ. [2009] Quantitation of protein, *Methods in enzymology*. 463:73-95.
- [19] Cahú TB, Santos SD, Mendes A, Córdula CR, Chavante SF, Carvalho LB, et al. [2012] Recovery of protein, chitin, carotenoids and glycosaminoglycans from Pacific white shrimp (*Litopenaeus vannamei*) processing waste, *Process Biochemistry*. 47:570-577.
- [20] Vázquez JA, Rodríguez-Amado I, Montemayor MI, Fraguas J, González MDP, Murado MA. [2013] Chondroitin sulfate, hyaluronic acid and chitin/chitosan production using marine waste sources: Characteristics, applications and eco-friendly processes: A review, *Marine drugs*. 11:747-774.
- [21] Yamada S, Sugahara K, Özbek S. [2011] Evolution of glycosaminoglycans: Comparative biochemical study, *Communicative & integrative biology*. 4:150-158.
- [22] Longas MO, Cheairs K, Puchalski MM, Park JI. [2011] Reliability of Fourier transform infrared spectroscopy in the characterization of human skin, *Advances in Biological Chemistry*. 1(24).
- [23] Pandian V, Thirugnanasambandan S. [2008] Glycosaminoglycans (GAG) from backwater clam *Marcia opima* (Gmelin), *Iranian Journal of Pharmacology & Therapeutics*. 7:147-151.
- [24] Wüthrich K. [1990] Protein structure determination in solution by NMR spectroscopy, *Journal of Biological Chemistry*. 265:22059-22062.
- [25] Pomin VH. [2013] NMR chemical shifts in structural biology of glycosaminoglycans, *Analytical chemistry*. 86:65-94.
- [26] Pomin VH. [2014] Solution NMR conformation of glycosaminoglycans, *Progress in biophysics and molecular biology*. 114:61-68.