Carbohydrate Polymers 98 (2013) 89–94

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation and characterization of mucilage polysaccharide for biomedical applications

G. Archana[a], K. Sabina[a], S. Babuskin[a], K. Radhakrishnan[a], Mohammed A. Fayidh[a], P. Azhagu Saravana Babu[a], M. Sivarajan[b], M. Sukumar[a,*]

[a] Centre for Biotechnology, A.C. Tech., Anna University, Chennai 600025, India
[b] Chemical Engineering Division, Central Leather Research Institute, Adyar, Chennai 20, India

ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form 5 April 2013
Accepted 17 April 2013
Available online 9 May 2013

Keywords:
Carbohydrates
Polysaccharide
Swelling index
Emulsifying capacity
Abelmoscus esculentus
Gracilaria corticata

ABSTRACT

In the present investigation, the polysaccharide/mucilage from waste of *Abelmoscus esculentus* by modification in hot extraction using two different solvents (Acetone, Methanol) were extracted, characterized and further compared with seaweed polysaccharide for their potential applications. The percentage yield, emulsifying capacity and swelling index of this mucilage were determined. The macro algae and okra waste, gave high % yield (22.2% and 8.6% respectively) and good emulsifying capacity (EC% = 52.38% and 54.76% respectively) with acetone, compared to methanol (11.3% and 0.28%; EC% = 50%) (PH = 7) while swelling index was greater with methanol than acetone extracts respectively. The infrared (I.R.) spectrum of the samples was recorded to investigate the chemical structure of mucilage. Thermal analysis of the mucilage was done with TGA (Thermal Gravimetric Analyzer) and DSC (Differential Scanning Calorimeter) which showed both okra and algal polysaccharide were thermostable hydrogels.

1. Introduction

Mucilage and gums are known as rich sources of polysaccharides since ancient times. They are widely used in the pharmaceutical & food industries as thickeners, water retention agents, and emulsion stabilizers, suspending agents, binders etc. Apart from their role in finished medicinal products, newer uses have been found in the preparation of cosmetics, textiles and paper industry. The okra plant *Abelmoschus esculentus* (L.) Moench, a native plant from Africa, is now grown in now commonly other areas such as Thailand, the Middle East India and the southern states of the USA. The optimum yield of okra is approximately estimated as 6.6 t ha^{-1}. Its water extracts are thick and slimy due to the presence of polysaccharides and are used to thicken soups and stews. Commercially these mucilage polysaccharides are known as hydrocolloids based on their wide range of functional properties. The okra or "gumbo" pod as a whole is used as vegetable for human consumption. But the upper part of the okra pods (upper crown head) are commonly removed or cut and thrown prior to cooking as waste (Al-Barak & El-Said, 2010). This part of okra pod also contains mucilage which gives its slimy viscous characteristic. Carbohydrates are mainly present in the form of mucilage (Sengkhamparn, Bakx, et al., 2009). Okra pods specially when heated produce more sticky mucus. This mucilage has a good potential for pharmaceutical applications such as diluents, binder, pharmaceutical excipients over synthetic excipients, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, film forming agents in transdermal and periodontal films and gelling agents in gel base for topical application due to ease of application for better percutaneous absorption. The main components of this mucilage are galactose (25%), rhamnose (22%), galacturonic acid (27%) and amino acids (11%) (Sengkhamparn et al., 2010). Previous studies showed okra fruit rhamnogalacturonans polysaccharide component increased cell proliferation. Savello et al. (1980), showed potential application of okra mucilage as a plasma replacement or blood volume expander. Similarly the phycocolloid polysaccharides obtained from some families of red macro algae (Rhodophyta), mainly Gracilariaceae and Gelidiaceae (Armisen, 1995) are widely used in pharmaceutical, cosmetics and food industry. Agar is essentially a mixture of the neutral polymer agarose, pyruvated agarose and sulfated galactans (Alves, Caridade, Mano, Sousa, & Reis, 2010). The ability of seaweeds to produce secondary metabolites of antimicrobial value, such as volatile components (phenols, terpenes) was well investigated in previous studies (Shukla, Kumar, Prasad, Reddy, & Jha, 2011). The objective of this study is to use a simple algal mucilage polysaccharide extraction procedure for extraction of mucilage polysaccharide from okra waste, thereby evaluate & compare the properties of crude mucilage polysaccharides for their potential industrial applications.

* Corresponding author. Tel.: +91 44 22358385; fax: +91 44 2235 0299.
E-mail address: sukumaractech@gmail.com (M. Sukumar).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.04.062

The aim of the present study was to use a different extraction method for okra mucilage polysaccharide (Distantina, Wiratni, Fahrurrozi, & Rochmadi, 2011) and to compare, investigate its properties with that of marine algal polysaccharide for their potential role in development of biopolymer for tissue engineering.

2. Materials and method

2.1. Sample collection and preparation

Seaweeds species (*Gracilaria corticata*) was collected from mandapam coast in Gulf of Mannar, Tamilnadu southeast coast of India at a latitude 9°45′N and longitude 79°0′E on low tide during December 2011. Collected samples were washed with tap water to remove epiphytes and other marine organisms. The samples were transported to the laboratory in sterile polythene bags. In the laboratory, samples were rinsed with tap water and were shade dried and powdered in a mixer grinder. Similarly okra bio waste (upper crown head) was collected from canteen, Anna University, Chennai, & rinsed with tap water and was shade dried. Dried okra wastes were broken to powder form in an electrical mixer. It was then sieved into particle sizes 0.5–4 mm and stored in glass bottles for further use. All reagents used in the experiment had analytical purity.

2.2. Extraction

The mucilage polysaccharides were extracted by the method followed by Distantina et al. (2011). 5 g of clean, dried seaweed and okra powder sample was soaked in distilled water for 15 min. After soaking, the water was separated from the seaweed by filtration. Firstly, a known amount of solvent (methanol, acetone; (1/50 g/mL)) was heated in a beaker as an extractor which emerged in a water bath equipped by a stirrer. If the temperature of solvent reached 85 °C (Distantina et al., 2011), the samples then were added into solvent, and the time of extraction started was counted. The speed of stirrer was set constant at 275 rpm. The constant ratio of seaweed weight to solvent volume (1/50 g/mL) was maintained by adding hot water. The extraction was stopped after 45 min. Filtrate was separated from residue using filter cloth and immediately poured into 3 volumes of cold (5 °C) acetone (90% w) which caused precipitation of polysaccharides. The precipitation was done for 30 min with stirring gently by hand. The precipitated polysaccharides were collected and oven dried at 50–60 °C to a constant weight. The experiments were carried out with different solvent such as acetone and some parameters namely the yield, chemical structure, swelling index, emulsifying capacity of the extracted polysaccharides were determined.

2.3. Chemical analysis

2.3.1. Organoleptic evaluation of polysaccharide

Both okra and seaweed polysaccharide were subject to qualitative evaluation based on the study of morphological and sensory profile by the organs of sense (skin, eye, tongue, nose and ear) and by macroscopic evaluation which includes color, odor, taste, size, shape and special feature, like touch, texture etc. The organoleptic characters of both the mucilage were found acceptable. The crude polysaccharides showed low solubility due to the presence of impurities, high molecular weight or large molecules and insoluble matters. Full solubility is beneficial from the view point of appearance and texture. Therefore, it is necessary to achieve the maximum solubilization in order to maintain the functionality in biopolymer development as reported earlier (Amid & Mirhosseini, 2012) and presented in Table 1

Table 1
Organoleptic evaluation of selected polysaccharide.

Parameter	*Abelmoscus esculentus*	*Gracilaria corticata*
Color	Cream	White
Odor	Honey	Odorless
Taste	Tasteless	Tasteless
Shape	Irregular	Irregular
Touch and texture	Hard and rough	Hard and rough
Solubility	Slightly soluble	Slightly soluble

2.4. Percentage yield

The percentage yield was calculated based on the amount of dry powder sample used for the extraction process and the amount of dry water soluble mucilage/polysaccharide obtained after the extraction. The percentage yield (mg/g dry extract) was calculated using the following formula as mentioned below,

$$\%\,\text{yield} = \frac{\text{wt. of dried mucilage obtained}}{\text{wt of power taken}} \times 100$$

2.5. Swelling index

In this study, 1.0 g of dry water soluble mucilage/polysaccharide was placed in a 100-mL stopper graduated cylinder. The initial volume of the dry mucilage/polysaccharide was measured. 2-mL of alcohol (95%) was added for good dispersion and then distilled/demineralized water was added to sufficient quantity to yield 100-mL of uniform dispersion. The viscous solution was stored at room temperature and the sediment volume of the swollen mass was noted after 24 h. The swelling ratio was calculated by determining the ratio of the swollen volume to the initial bulk volume using the formula.

$$S = \frac{V_2}{V_1}$$

where S is the swelling index;V_2 is the volume occupied by the gum prior to hydration; V_1 is the volume occupied by gum after hydration.

2.6. Emulsifying capacity

Mucilage powder was used to determine the emulsion capacity. The samples were accurately weighed (1.0 g), dissolved in 50 ml distilled water, and added with 50 ml refined oil (Thanatcha & Pranee, 2011). Then the emulsion was prepared by homogenizing the above mixture for 1 min and centrifuged with 4100 × g for 5 min. Finally measured the height of emulsified layer compared with the height of whole layer and calculated the emulsion capacity by the following equation.

$$\text{Emulsifying capacity}\,\% = \frac{\text{Height of emulslfied layer}}{\text{Height of whole layer}} \times 100$$

2.7. Infrared spectroscopy (IR)

A potassium bromide disk of each of the dried mucilage was prepared, and the infrared spectra recorded (Perkin-Elmer 720) between 4000 and 650 cm^{-1}. By referring to wavelengths of polysaccharides from previous research, the result showed the chemical groups corresponding the wave number of bonds cm^{-1}. Apart from usual bands for hydroxyl (3500 cm^{-1}) and ester carbonyl (1730 cm^{-1}) groups, Nitrogen of protein (1550 cm^{-1}), amide deformation (1630 cm^{-1}) and ether group (1220 cm^{-1}) can be distinguished. The IR spectra of crude seaweed extracts were also determined.

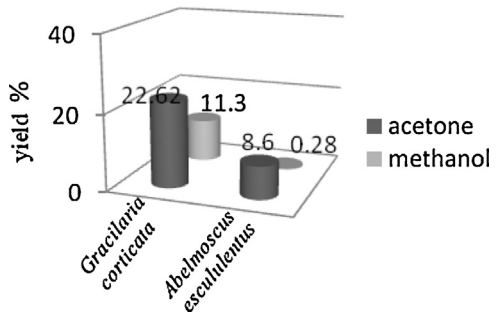

Fig. 1. Percentage yield of *G. corticata* and *A. esculentus* polysaccharide with methanol and acetone.

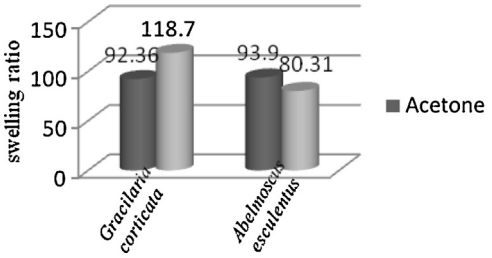

Fig. 2. Swelling index of *G. corticata* and *A. esculentus* polysaccharide with methanol and acetone.

2.8. Thermal analysis of the samples

Thermogravimetry (TG) and Differential scanning calorimeter (DSC) techniques were used to analyze the thermal stability of okra and *Gracilaria corticata* polysaccharides. The measurements were performed using a thermo balance DSC Q10 v9.0 build 275 Differential scanning calorimeter and SDT Q600 in nitrogen atmosphere (250 ml/min) in a programmed temperature range from 30 to 600 °C at a heating rate of 10 °C/min. Sample weights between 5 and 10 mg were placed in a platinum pan.

3. Results and discussion

3.1. Percentage yield

The different extraction technique used for extraction of okra polysaccharide yields are shown in Fig. 1. The yield of okra polysaccharide (8.6% (acetone) and 0.28% (methanol)) with this newer protocol was nearly similar to previously reported yield % by Sengkhamparn, Verhoef, et al., 2009, and higher compared to previously reported yield by Kumar et al. (2009). The yield of gracelaria polysaccharide seems to be similar as earlier reported by Andriamanantoanina et al. (2007) but less than that reported by Distantina et al. (2011). It seems that both acetone and methanol could not be better solvent for this type method of polysaccharide extraction seaweeds than KOH or water. However, from the present experiment a general conclusion could be given that the newer extraction protocol was suitable for seaweed but did not improve the yield of okra polysaccharides. The extraction procedure showed no significant effect on yield of okra as well as gracelaria polysaccharides (Barros et al., 2013). These results showed that the decrease in yield may be due to increased temperature, solvents used or polymer degradation which needs further detailed investigation.

3.2. Swelling index

The swelling characteristic of both acetone and methanol extract polysaccharide was studied and shown in Fig. 2. Methanol extract polysaccharide showed higher swelling ratio. Acetone extracts of both okra and seaweed showed nearly similar swelling properties. Even in methanol extract, seaweed polysaccharide exhibited good swelling capacity compared to okra (Zhanga et al., 2010). The higher swelling capacity of seaweed mucilage showed their higher water-holding capacities (Sikareepaisan, Ruktanonchai, & Supapho, 2011). The increase in swelling increases the surface area, surface wettability and consequently, water penetration to form biofilm matrices with higher hydrophilic nature which could be easily biodegradable. This may also be due to presence of hydrophilic groups (such as hydroxyl groups) as determined by IR data (Zhao et al., 2009). On the other hand okra polysaccharide showed lower swelling index than *Gracilaria corticata*. This may be attributed to various factors such as presence of impurities (crude sample), hydration positions, extraction procedure and environmental conditions. In general a better swelling property of the hydrogel enhances pore characteristics as well as hydrophilic nature of the scaffold with open pore structure essential for uniform cell distribution and proper diffusion of nutrients for tissue engineering applications. It seems unlikely that the varying swelling capacities of the polysaccharides cause the change of morphology such as poor mechanical strength, low surface area when used for the scaffold development. The decrease of pore size may causes capillary phenomenon and increases some water absorption which results in uncontrolled solute diffusions. During cell cultivation, 3D porous scaffolds need to have appropriate strength for cells attachment. Therefore scaffolds' diameter could be optimized to enable some swelling before implantation as given by Bernke Papenburg (2009). Also higher levels of cross linking may result from lower swelling capacities. In the development of tissue scaffolds, the maintenance of porous structure and low interfacial tension toward aqueous fluid is necessary which helps in creating a hydrophilic network appropriate which could strengthen cells-attachment. The lower swelling capacity of okra showed that when used for hydrogel may lower the surface area of polymers and induces loss of porosity during biopolymer formation. Higher the amount of water imbibed (hydration capacity) within the polysaccharides, higher the diffusion properties of a solute through the hydrogel. Higher water holding capacity could also influence the cross-linking of the hydrophilic groups which results in increase in hydrophobicity of polymers. As the cross linking density increases, there is a subsequent increase in the hydrophobicity and a corresponding decrease in the stretchability of the polymer network (Prommakool, Sajjaanantakul, Janjarasskul, & Krochta, 2011.). Therefore, determination of WHC (Water Holding Capacity) of polysaccharides for hydrogel formation is an important criterion for characterizing the hydrogel for biomedical applications. It seems unlikely that the varying swelling capacities of the polysaccharides cause the change of morphology when used for the scaffold development. The scaffolds' diameter could be optimized to enable some swelling before implantation as given by Bernke Papenburg (2009). This suggests that the crude mucilage polysaccharide needs to be purified and purest forms could be used for biopolymer formation. Swelling is a primary mechanism in diffusion controlled release dosage form. The use of okra mucilage as binder/disintegrant in drug delivery had been well established in previous research by various authors. Okra mucilage and modified okra mucilage were successfully used as coating materials in combination with HPMC (Hydroxy propyl methyl cellulose) K15M to deliver model drug diclofenac sodium to colon for chronotherapy of arthritis. The drug release was extended for 24 h in colon. But higher degree of swelling may result in excessive hydration thereby resulting in a reduction of interaction between mucoadhesive polymers owing to less efficacious and lowest bioadhesive strength. Thus it may be envisaged that okra waste mucilage rich in protein and for

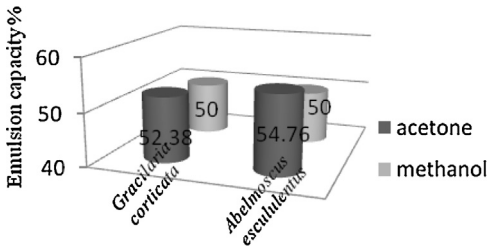

Fig. 3. Percentage emulsion capacity of *G. corticata* and *A. esculentus* with acetone and methanol.

scaffold with controlled porosity and *Gracilaria corticata* polysaccharide could be used for development of biofilm scaffolds with good mucoadhesive capacity which would form hydrophilic polymers that would leach out with more pores and channels for the drug/cells to diffuse out of them owing their potential for tissue engineering.

3.3. Emulsifying capacity

The capacity of protein to enhance the formation and stabilization of emulsions is important for many applications in food products like cake, coffee whiteners and frozen desserts. In these products, varying emulsifying and stabilizing capacity are required because of different compositions and stresses to which these products are subjected. Both acetone and methanol extracts of seaweed and okra showed good emulsion capacity by clear non-separation of liquid layers after centrifugation shown in Fig. 3. The emulsion capacity of okra mucilage powder was analogous to that of Locust bean gum (52%) but less than xanthan gum (94%), Ocimum canum seeds (74.41%). This emulsion capacity can be related with stability of product such as salad dressing because it can enhance formation of small droplets and reduce the rate at which droplets coalesce (Kayalvizhi, Subramanian, Anantharaman, & Kathiresan, 2012). This emulsion capacity of okra and gracelaria showed that they can form structurally stable and entirely edible bio films with PEG, chitosan or xanthan gum as that of locust bean gum for potential applications in food packaging industries as reported in previous studies. The findings also suggest both polysaccharide may play a vital role as an emulsion system singly or in blended form to maintain homogenousity of various scaffold solutions in tissue engineering like DMPC (1,2-Dimyristol-sn-Glycero-3-Phosphocholine), which can prevent chemical changes between different materials and result in the development of low-cost, biocompatible, and antimicrobial bio scaffolds with desirable properties such as scaffold morphology, controlled porosity, enhanced cell attachment and survival as previously reported (Maciel et al., 2008).

3.4. Infrared spectroscopy (IR)

The IR spectrums of crude mucilage extracts are shown in Fig. 4. This technique is considered as an useful information for partial characterization of seaweeds (Madhaiyan & Rajasulochana, 2012), such as in the determination of sulfate and 3,6-anhydrogalactose contents in seaweed polysacharides (Ordonez et al., 2012). The seaweed mucilage polysaccharide exhibited more or less common constant peaks. The occurrence of peak at the 2925 cm^{-1} signifies C—H asymmetric stretching. 3400–3423 (OH stretching) and 2922–2926 cm^{-1} (CH stretching) were also detected. Strong and very sharp peak at 1646 cm^{-1} is observed in the present study for amide I deformation/C=O asymmetric stretching. Band absorbance at 617,889,932 due to occurrence of 3,6-anhydro-D-galactose and characteristics of agarocolloids in the crude extracts. The band at 890 cm^{-1} is typical of β-D-galactopyranose residues of agar. So these bands of IR for *Gracilaria corticata* confirms it a phycocolloid polysaccharide (Fernández et al., 2003). Similarly okra mucilage showed bands at 3402.5, 2928.0, 2366.9, 2345.4, 1617.6, 1419.9, 1319.9, 1281.3, 1195.9, 1096.1, and 669.6. Apart from the usual bands for hydroxyl (915–955 cm^{-1}) and ester carbonyl (1730 cm^{-1}) groups, protein (carbonyl stretch 1660–1680 cm^{-1}), amide deformation (1617 cm^{-1}) and phosphate (1260–1280 cm^{-1}) can be distinguished. The peaks at 2925 and 2854 cm^{-1} is the characteristic band for the C—H stretching vibration from CH and CH2 in cellulose and hemicelluloses components. Polymers with hydrophilic groups, such as carboxyl and hydroxyl groups, bind strongly to the oligosaccharide chains of the mucous layer. This shows the okra waste polysaccharide is neither starch nor cellulose, but has some peptide cross-links and some amino sugars (Mishra, Clark, & Sunita, 2008). From the IR results it could be inferred that the numerous amount of hydrogen bond forming groups i.e., carboxyl and hydroxyl groups in both these polysaccharides gives a hint for their good bioadhesive property singly or in combination polysaccharides like alginate sulfate (SALG) toward biofilm formation and drug delivery systems. This also shows Viscous substances of diverse polymeric compositions can be developed for various applications such as in opthalmology, dermatology, and orthopeadics. Many actives can be released through such bioadhesives, as steroids, anti-inflammatory agents, pH sensitive peptides and small proteins such as insulin, and local treatments to alleviate pain in the buccal cavity. From the IR data we can conclude that the kappa carageenan can form stronger gels with okra waste polysaccharide which is rich in protein, lignin by cross linking with Ca^{2+} ions, Ba^{2+} and Sr^{2+} (Deng, He, Zhao, & Yang, 2007) similar to guar gum.

3.5. Thermal analysis of the samples

The TGA curves of both the samples were recorded and *Gracilaria corticata* showed significant decomposition compared to okra polysaccharide as given in Fig. 5. Okra polysaccharide did not show any significant weight loss below 300 °C. The polysaccharide showed weight loss of 51.15% at 362 °C. The weight loss of okra was below 200 °C (Prajapati, Jani, Moradiya, & Randeria, 2013) was minimal which may be due to increased inorganic materials or impurities in the crude sample due to handling or human error. This showed that okra polysaccharide has good thermal stability (Mishra & Sunita, 2007). In case of *gracelaria*, decomposition takes place at two different temperatures 289 °C and 817 °C with weight loss of 24.15% and 24.67% showing increased inorganic substances than okra (Stephanie et al., 2010). This may be due to methylation which releases inorganic materials like oxides, carbonaceous residues.

The output graph of DSC for okra and *Gracilaria corticata* polysaccharide showed melting with an onset temperature of 37.51 °C and a peak of 62.32 °C with an associated enthalpy change of 86.63 J/g and 40.31 °C and a peak of 77.88 °C (Enthalpy = 144.3 J/g). The melting occurs over a large range of temperatures which could be impurities present in the sample; it might be due to amorphous nature or vaporization of the water indicating the presence of hydrophilic groups in the crude samples. Also, an exothermic transition has occurred in okra, beginning at 221.41 °C, running through a peak of 225.48 °C with a small enthalpy change of 7.004 J/g. There was also a third transition observed in okra at 251.38 °C, running through a peak of 265.85 °C with a small enthalpy change of 94.77 J/g. According to references, temperature ranges between 220 and 310 °C which was associated to the thermal depolymerisation of hemicellulose, pectin and the cleavage of glycosidic linkages of cellulose. An exothermic transition has occurred with *Gracilaria corticata* (Fan et al., 2011), running through

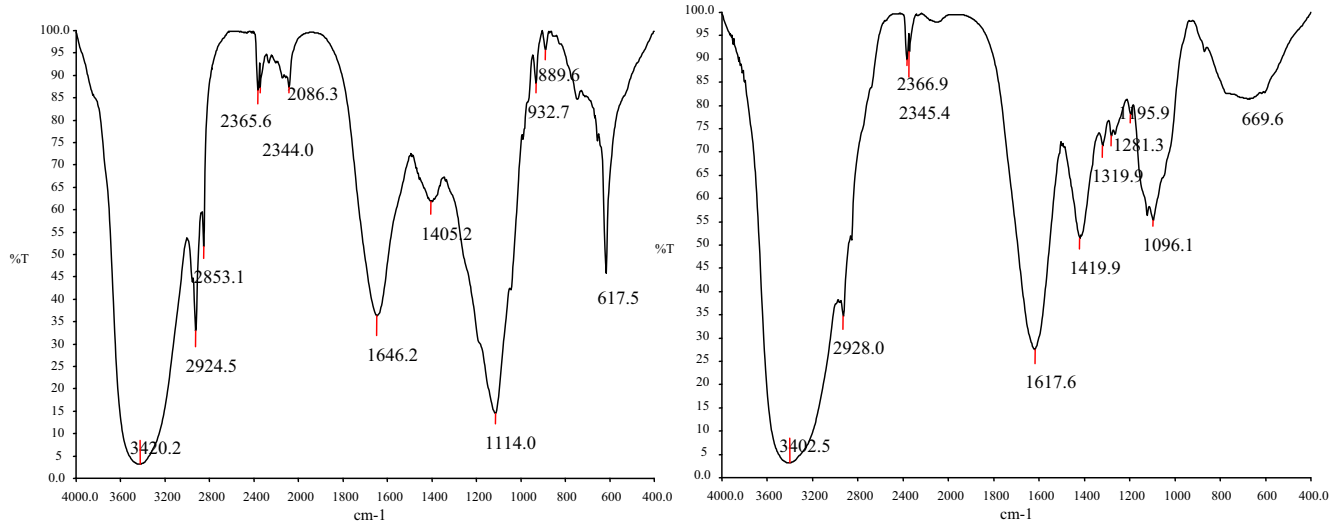

Fig. 4. Infra red spectra of seaweed and okra mucilage polysaccharide.

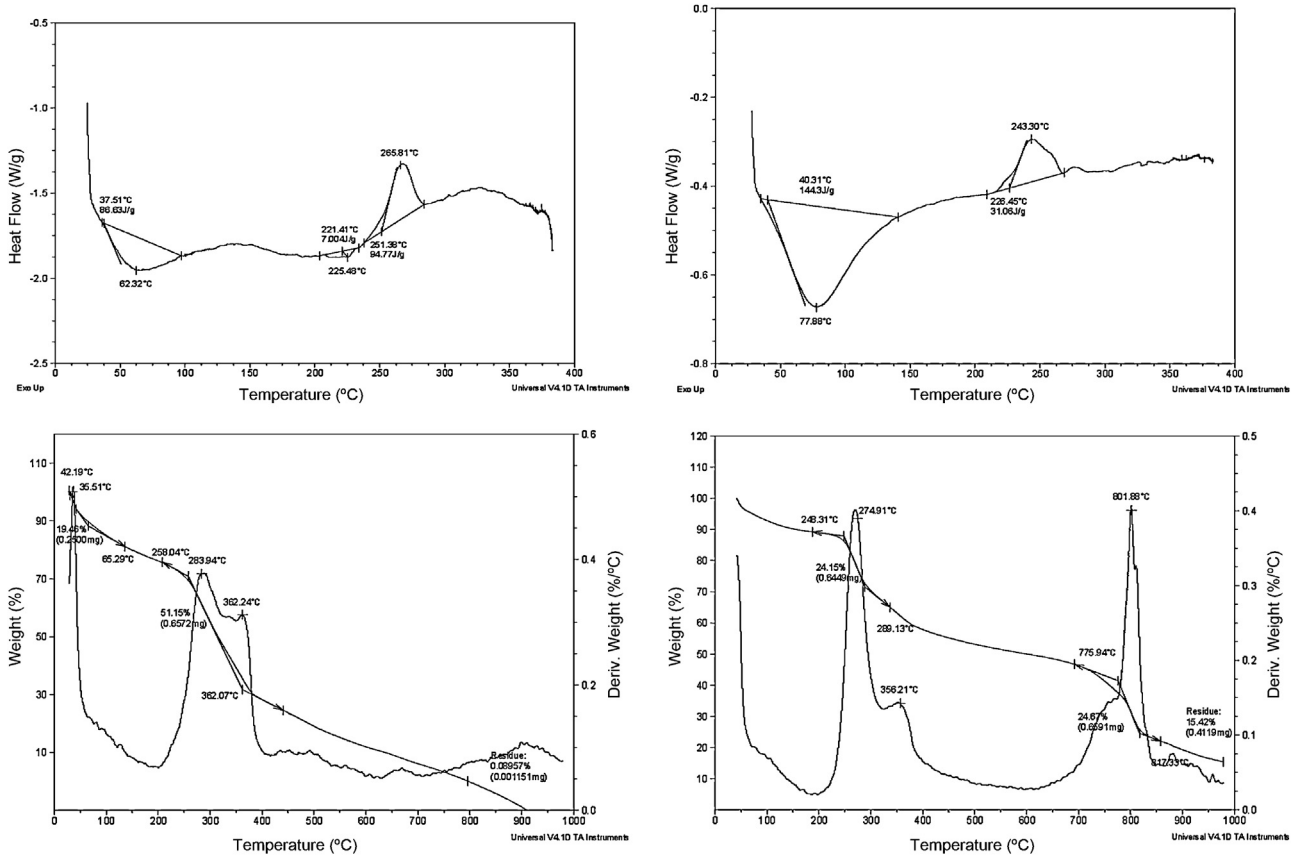

Fig. 5. DSC and TGA curves of okra and seaweed polysaccharide.

a peak of 243.30 °C with a small enthalpy change of 31.06 J/g. These finding suggest that both okra and seaweed polysaccharide are highly thermostable and could be used in the formation of biomaterials for various industrial purposes such as to improve bake-stability in cakes, improving freeze-thaw stability, preventing crystal growth, stabilizing suspensions or emulsions in paints, and encapsulation.

4. Conclusion

As a first conclusion, it can be showed that the protocol proposed for successive extractions of mucilage polysaccharides from okra biowaste was simpler and but showed less significant effects on yield when compared to methods reported in the previous literature. The results of the physicochemical parameters of the mucilage

showed all the characteristics of okra and seaweed mucilage as a good pharmaceutical agent. Both okra and seaweed mucilage polysaccharide showed good swellable property. The emulsion capacity of both okra and seaweed polysaccharide similar locust bean gum shows that they have good gelling, stabilizing or thickening properties to be applied in both food and pharmaceutical industries. From the DSC experiments it can be shown that polymers melt on heating and recrystallise cooling proving they are thermosets which can be processed and molded. Okra polysaccharide could be used to improve mechanical performances of alginate-based biofilms is also of interest. Thus the blending of these two natural polysaccharides by chemical modification to create specific interactions with the other one can produce highly compatible, very homogeneous and biodegradable alginate-based biofilms, a versatile biopolymer for tissue engineering. All the above results suggest that these two plants extract natural polysaccharides showed promising potential for biopolymer development for various food and pharmaceutical industries. This shows a new trend for using agricultural wastes from food processing industries for development of cheaper, biocompatible and biodegradable biopolymer for various purposes such tissue engineering as well as a mean for wastes disposal for the benefit of environmental pollution control. The mucilage pectic polysaccharide was not of animal origin and less prone to cause inflammatory response in a patient which makes the pectin-like polysaccharide suitable for use in various biomedical applications. In the solid state pectins exist in right-handed helices stabilized by intramolecular and intermolecular hydrogen bondings and tend to associate to form stiff rods or segmented rods in solution. This effect of viscosity has been attributed to bridging between suitably positioned carboxyl groups leading to the formation of supramolecular structures. Thus blending of high molecular okra polysaccharide with agar polysaccharide can produce more stable rigid hydrogels for tissue engineering applications. The results of the present study showed that *G. corticata* and okra waste contains rich sources of polysaccharides which could be isolated and further screened for different kinds of biological activities, based on their potential therapeutic applications.

References

Al-Barak, A. B. S., & El-Said, S. M. (2010). The use of some food industrial by products for waste water purification. *Research Journal of Environmental Sciences*, *4*, 566–572.

Alves, A., Caridade, S. G., Mano, J. F., Sousa, R. A., & Reis, R. L. (2010). Extraction and physico-chemical characterization of a versatile biodegradable polysaccharide obtained from green algae. *Carbohydrate Research*, *345*, 2194–2200.

Amid, B. T., & Mirhosseini, H. (2012). Effect of different purification techniques on the characteristics of hetero polysaccharide-protein biopolymer from durian (*Durio zibethinus*) seed. *Molecules*, *17*(9), 10875–10892.

Andriamanantoanina, H., Chambat, G., & Rinaudo, M. (2007). Fractionation of extracted Madagascan *Gracilaria corticata* polysaccharides: Structure and properties. *Carbohydrate Polymers*, *68*, 77–88.

Armisen, R. (1995). World-wide use and importance of *Gracilaria*. *Journal of Applied Phycology*, *7*, 231–243.

Barros, F. C. N., Silva, D. C., Sombra, V. G., Maciel, J. S., Feitosa, J. P. A., Freitas, A. L. P., et al. (2013). Structural characterization of polysaccharide obtained from red seaweed *Gracilaria caudata* (J Agardh). *Carbohydrate Polymers*, *92*, 598–603.

Deng, C. M., He, L. Z., Zhao, M., & Yang, D. (2007). Biological properties of the chitosan–gelatin sponge wound dressing. *Carbohydrate Polymers*, *69*, 583–589.

Distantina, S., Wiratni, Fahrurrozi, M., & Rochmadi. (2011). Carrageenan properties extracted from *Eucheuma cottonii*, Indonesia. *World Academy of Science, Engineering and Technology*, *54*, 738–742.

Fan, Y., Wang, W., Song, W. M., Chen, H., Teng, A., & Liu, A. (2011). Partial characterization and anti-tumor activity of an acidic polysaccharide from *Gracilaria lemaneiformis*. *Carbohydrate Polymers*, *85*, 157–163.

Fernández, J. P., Vazquez, J. A. R., Tojo, E., & Andrade, J. M. (2003). Quantitation of κ-, ι- (iota) and λ-carrageenans by mid-infrared spectroscopy and PLS regression. *Analytica Chimica Acta*, *480*, 23–37.

Kayalvizhi, K., Subramanian, V., Anantharaman, P., & Kathiresan, K. (2012). Antimicrobial activity of seaweeds from the Gulf of Mannar. *International Journal of Pharmaceutical Applications*, *3*(2), 306–314.

Kumar, R., Patil, M. B., Patil, S. R., & Paschapur, M. S. (2009). Evaluation of disintegrating properties of *Abelmoschus esculentus* mucilage. *International Journal of PharmTech Research*, *1*(2), 241–246.

Maciel, J. S., Chaves, L. S., Souza, B. W. S., Teixeira, D. I. A., Freitas, A. L. P., Feitosa, J. P. A., et al. (2008). Structural characterization of cold extracted fraction of soluble sulfated polysaccharide from red seaweed *Gracilaria birdiae*. *Carbohydrate Polymers*, *71*, 559–565.

Madhaiyan, B. S., & Rajasulochana, N. (2012). Chemical and spectral characterization of cell wall polysaccharide of *Gracilaria edulis* (Gmelin). *Journal of Theoretical and Experimental Biology*, *8*(3 and 4), 147–151.

Mishra, A., Clark, J. H., & Sunita, P. (2008). Modification of okra mucilage with acrylamide: Synthesis, characterization and swelling behavior. *Carbohydrate Polymers*, *72*, 608–615.

Mishra, A., & Sunita, P. (2007). Polyacrylonitrile-grafted okra mucilage: A renewable reservoir to polymeric materials. *Carbohydrate Polymers*, *68*, 95–100.

Ordonez, E. G., Jimenez-Escrig, A., & Ruperez, P. (2012). Molecular weight distribution of polysaccharides from edible seaweeds by high-performance size-exclusion chromatography (HPSEC). *Talanta*, *93*, 153–159.

Papenburg, B. (2009). . *Design strategies for tissue engineering scaffolds* (vol. 239) The Netherlands: University of Twente. PhD thesis, ISBN: 978-94-9012-3.

Prajapati, V. D., Jani, G. K., Moradiya, N. G., & Randeria, N. P. (2013). Pharmaceutical applications of various natural gums, mucilages and their modified forms. *Carbohydrate Polymers*, *92*, 1685–1699.

Prommakool, A., Sajjaanantakul, T., Janjarasskul, T., & Krochta, J. M. (2011). Whey protein–okra polysaccharide fraction blend edible films: Tensile properties, water vapor permeability and oxygen permeability. *Journal of the Science of Food and Agriculture*, *91*, 362–369.

Savello, P. H., Martin, F. W., & Hill, J. M. (1980). Nutritional composition of okra seed meal. *Journal of Agricultural and Food Chemistry*, *28*, 1163–1166.

Sengkhamparn, N., Bakx, E. J., Verhoef, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J. (2009). Okra pectin contains an unusual substitution of its rhamnosyl residues with acetyl and alpha-linked galactosyl groups. *Carbohydrate Research*, *344*, 1842–1851.

Sengkhamparn, N., Sagis, L. M. C., de Vries, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J. (2010). Physicochemical properties of pectins from okra (*Abelmoschus esculentus* (L.) Moench). *Food Hydrocolloid*, *24*, 35–41.

Sengkhamparn, N., Verhoef, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J. (2009). Characterisation of cell wall polysaccharides from okra (*Abelmoschus esculentus* (L.) Moench). *Carbohydrate Research*, *344*, 1824–1832.

Shukla, M. K., Kumar, M., Prasad, K., Reddy, C. R. K., & Jha, B. (2011). Partial characterization of sulfohydrolase from *Gracilaria dura* and evaluation of its potential application in improvement of the agar quality. *Carbohydrate Polymers*, *85*, 157–163.

Sikareepaisan, P., Ruktanonchai, U., & Supapho, P. (2011). Preparation and characterization of asiaticoside-loaded alginate films and their potential for use as effectual wound dressings. *Carbohydrate Polymers*, *83*, 1457–1469.

Stephanie, B., Eric, D., Sophie, F. M., Christian, B., & Yu, G. (2010). Carrageenan from *Solieria chordalis* (Gigartinales): Structural analysis and immunological activities of the low molecular weight fractions. *Carbohydrate Polymers*, *81*, 448–460.

Thanatcha, R., & Pranee, A. (2011). Extraction and characterization of mucilage in *Ziziphus mauritiana* Lam. *International Food Research Journal*, *18*, 201–212.

Zhanga, Z., Wang, F., Wang, X., Liud, X., Houb, Y., & Zhang, Q. (2010). Extraction of the polysaccharides from five algae and their potential antioxidant activity in vitro. *Carbohydrate Polymers*, *82*, 118–121.

Zhao, Q. S., Xia Ji, Q., Ke Xing, Yan Li, X., Sheng Liu, C., & Chen, X. G. (2009). Preparation and characteristics of novel porous hydrogel films based on chitosan and glycerophosphate. *Carbohydrate Polymers*, *76*, 410–416.