Biophysical-chemistry Characterization of Alginate in Brown Algae Species Sargassum dupplicatum

Dang Xuan Cuong1, *, Dang Thi Thanh Tuyen2, Do Thuy Kieu2

1Organic Material from Marine Resource, Nhatrang Institute of Technology Application and Research, Vietnam Academy of Science and Technology, Nha Trang, Vietnam
2Faculty of Food Technology, Nha Trang University, Nha Trang, Vietnam

Email address: cuong mails@yahoo.com.vn (D. X. Cuong), thanhtuyen151809@gmail.com (D. T. Tuyen), kieuthuy8192@gmail.com (Do T. Kieu)

*Corresponding author

To cite this article:
Dang Xuan Cuong, Dang Thi Thanh Tuyen, Do Thuy Kieu. Biophysical-chemistry Characterization of Alginate in Brown Algae Species Sargassum dupplicatum. World Journal of Food Science and Technology. Special Issue: Marine Bio-Polymer: Bio-Activity, Extraction and Application. Vol. 4, No. 1, 2020, pp. 17-22. doi: 10.11648/j.wjfst.20200401.13

Received: March 17, 2020; Accepted: April 2, 2020; Published: April 29, 2020

Abstract: Alginate is a high-value biopolymer, exists in brown algae, and applied widely in numerous fields, for example, food, functional foods, and pharmaceutics. The study focused on the biophysical-chemistry characterization of alginate in brown algae species Sargassum dupplicatum grown commonly in Vietnam under the effect of NaCl, KCl, MgCl2, chitosan, carrageenan, and ethanol in different physical condition. Antioxidant activity of alginate and their compound was also studied. The results showed that Na_Alg, K_Alg, and Mg_Alg disperse the net style in the water. Ca_Alg, chitosan_Alg, and Carrageenan_Alg absorbed water and swelled for forming a sphere, yarn, and yarn in the water, respectively. Chitosan_Alg and Carrageenan_Alg precipitated faster in ethanol, compared to Na_Alg, K_Alg, and Mg_Alg. 20% of ethanol did not cause the precipitation of Alginate salt, Chitosan_Alg, and Carrageenan_Alg for 30 minutes. Alginate salt, Chitosan_Alg, and Carrageenan_Alg were full precipitated for 30 minutes when using ethanol concentration was more than 80%. Chitosan_Alg and Carrageenan_Alg occurred the precipitation in 20% of ethanol for 30 minutes. Total antioxidant activity and reducing power activity of chitosan_Alg got the highest value (a and b, respectively), compared to Carrageenan_Alg, Na_Alg, K_Alg, and Mg_Alg. The antioxidant activity difference in a group of Na_Alg, K_Alg, and Mg_Alg, and a group (Chitosan_Alg and Carrageenan_Alg) did not happen. The difference only occurred between the two groups.

Keywords: Alginate, Chitosan, Carrageenan, Antioxidant, Ethanol

1. Introduction

Alginate is calcium, magnesium, and sodium salts of alginic acid found commonly in the cell wall of brown algae. Alginate is an unbranched biopolymer consisting of (1→4)-linked β-d-mannuronic and α-l-guluronic acid residues. The structure, the bioactive, and the content of alginate, as well as mannuronate to guluronate ratio in alginate, depended on various algae species, habituation, and season. The mannuronate/guluronate ratio of alginate in species Cystoseira barbata [1], Laminaria digitata [2], Macrocystis pyrifera, and Laminaria hyperborea [3] was 0.59 (37%, mannuronic acid, and guluronic acid, respectively), 1.2, 1.6, and 0.45, respectively. The viscosity and average molecular weight of alginate varied from 31 to 5,500 (cm³/g) and 5,100 to 2,700,000 g/mol, respectively [4]. They can absorb water taking up 200–300 times, compared to their weight for forming the gelation that depends on alginate concentration [5] and temperature [6]. Therefore, they are useful in the food industry, medicine, cosmetics, and biotechnology. Different types of alginate play various roles, for example, emulsifiers, gelling agents, thickener, stabilizer, coating agent, and membrane agent. Alginate is also used in the immobilization of enzyme and pigment, in inks, in material (bioplastics, dentistry, prosthetics, and lifecasting).

Alginate possesses value-high bioactive consisting of cytotoxicity, antibacterial, antioxidants [2, 7], heartburn [8], and gastroesophageal reflux disease [9-11]. Their bioactive
was improved when they combined to active polymer, for example, chitosan [12], carrageenan [13], polyphenol [14], and drugs [15]. Previous studies show that free radicals and oxidation stress are one of the reasons leading to the disease of reflux esophagitis, Barrett’s esophagus [16; 17], and inflammation [18] in humans. Therefore, antioxidant activity is one of the values and desirable activities of alginate.

Brown algae species *Sargassum duplicatum* found commonly grown in numerous areas in the world, for example, Pacific ocean [19], Indian ocean [20]. The previous studies show the content and characterization of alginate extracting from the species, but mainly the extraction yields [21], typical alginate, the morphology, chemical element, and functional groups [22]. The non-notices presented alginate of the species in Vietnam.

Thus, the study presented the characterization of physical, chemical, and biological (total antioxidant activity, and reducing power activity) of alginate extracting from *Sargassum duplicatum* commonly grown in Vietnam.

### 2. Material and Methods

#### 2.1. Material

Alginate (Alg) from brown algae *Sargassum duplicatum* commonly found in Vietnam was extracted in HCl solution (pH 2) for 24 hours and filtering through the membrane. The residues were soaked in Na$_2$CO$_3$ solution (pH 9) at 50°C for 4 hours according to the solvent to algae of 40/1 (v/w) and filtering the membrane. Alginic acid in the filtrate was precipitated in HCl solution (pH 2) and filtered. Alginic acid was continuously neutral cleaned by using Na$_2$CO$_3$ solution, precipitated by using 96% ethanol and dried at 60°C for 30 minutes.

κ – carrageenan was extracted from red algae *Kappaphycus alvarelli* at 80°C for 2 hours with aqueous according to the aqueous to red algae ratio of 30/1 (v/w). After extraction, the supernatant was filtered through the membrane, precipitated in 96% ethanol, and dried at 60°C for 20 minutes for collecting κ – carrageenan. The viscosity and gel strength of κ – carrageenan corresponded to 67.5 mPa/s and 218.9 g/cm$^2$. The SO$_3$ content of κ – carrageenan was 21.4±0.2%.

Chitosan was prepared according to the enzyme-assisted chemical method as the description in [23] with a light adjustment. Shrimp heads waste was macerated in the distilled water at 50°C for 2 hours and collecting the residue through the filtration. The residue was then soaked with the enzyme/residue ratio of 2% (pH 8) at 55°C for 8 hours. The mixture was continuously adjusted pH by using 1N NaOH and stopped the reaction by the heat at 90°C for 05 minutes. The protein in the residue was continuously removed by 2% of (w/v) NaOH at room temperature with the solid/liquid ratio of 1/5 (w/v) for 12 hours. After the protein movement, the demineralization in the deproteinized residue was by soaking at room temperature for 12 h in 4% HCl solution with the solid/liquid ratio of 1/5 (w/v) for collecting the chitin. Chitin was soaked in 50% (v/v) NaOH at 65°C for 20 hours and purified by dissolving in 1% lactic acid, and the precipitation of chitosan was by 4% NaOH at pH 10. The purified chitosan was finally cleaned by using 96% ethanol and dried by the freeze-drying method.

All chemicals using in the analysis were from Sigma - Aldrich, except for distilled water and 96% ethanol of Vietnam.

#### 2.2. Sample Preparation

10 g of alginate was in turn dissolved in difference solutions, for example, 5% of NaCl, 5% of KCl, 5% of CaCl$_2$, 5% of MgCl$_2$, 1% of chitosan, 1% of carrageenan for forming the mixture of sodium alginate (Na$_3$ Alg), potassium alginate (K$_3$ Alg), magnesium alginate (Mg$_3$ (Alg)), chitosan alginate (chitosan Alg), and carrageenan alginate (carrageenan Alg), respectively. These mixtures were filtered and evaluated on the characterization of physic chemistry and biology (antioxidant activity). For the evaluation of the precipitation of Alg mixture in ethanol, the adjustment of ethanol concentration in these mixtures corresponded to 20%, 40%, 60%, 80% và 100%, respectively.

#### 2.3. Evaluation of Physical Characterization

The phenomenon observation occurred in the reaction between alginate, different salts (sodium, potassium, magnesium, and calcium), and polymers (chitosan and carrageenan) for evaluating physical characterization (morphology).

#### 2.4. Evaluation of Chemical Characterization

One ml of 0.8 M sodium hydroxide mixed 01 ml of alginate solution (1mg/1ml) and after 5 minutes, neutral by 120 ml of 2.25 M citric acid. The mixture then added to 40 ml of DMMB reagent (Dimethylmethylene Blue Assay), vortexed, and kept for 45 minutes at room temperature. The absorbance measurement of the mixture was at the wavelength of 520 and 650 nm for the calculation of alginate content based on the ratio of 520:650 nm [24] that used in the determination of non-precipitating alginate ratio in ethanol.

#### 2.5. Evaluation of Biological Characterization

The equations are an exception to the prescribed specifications of this template. You will need to determine whether or not your equation should be typed using either the Times New Roman or the Symbol font (please no other font).
2.5.1. Total Antioxidant Activity
Total antioxidant activity was determined as in [25], basing to the metabolism of Mo(VI) to Mo(V) with ascorbic acid standard, and the absorbance measurement at the wavelength of 695 nm.

2.5.2. Reducing Power Activity
Reducing power activity was determined to base to the metabolism of Fe3+ to Fe2+ with FeSO4 standard, described in [26]. The absorbance measurement was at 655 nm.

2.6. Data Analysis
All experiments were in triplication (n=3). Statistic analysis was by using the software MS. Excel 2010.

3. Results and Discussion

3.1. Physical Characterization of Alginate
K_Alg, Na_Alg, and Mg_Alg dispersed and formed a distributing network in the water leading the viscosity characterization of the solution that was dependent on the concentration of ion metal (Na, K, and Mg). The viscosity increased following the non-linear model as the concentration increase of Na, K, and Mg. Ca_Alg in Ca2+ solution, Alg in 1% of chitosan at pH 4.5, and Alg in 1% of carrageenan at pH 7 formed the fibrous or spherical shapes that depended on the injection tip. Alg, Chitosan_Alg, and Carrageenan_Alg did not disperse in the water but absorbed and swelled in the water. The absorbency tendency of the Na+ hydrogels in salt solutions was stronger than Ca2+ hydrogels and K+ hydrogels, as in [27]. Moreover, the LD50 of Na_Alg (mg/kg body weight) was higher than Ca_Alg and K_Alg. Therefore, different Alg salts led the various applications, for example, Na_Alg in the beverage, the food, and the pharmaceutics, Ca_Alg in material, and K_Alg in the mask and the cosmetics.

3.2. Chemical Characterization of Alginate

3.2.1. The Precipitation of Alg Mixture in Ethanol
The table is as follows: All Alg mixture (Na_Alg, K_Alg, Mg_Alg, and Ca_Alg) did not precipitate in 20% of ethanol, except for chitosan_Alg and carrageenan_Alg that formed the precipitation in 20% of ethanol. When the ethanol concentration increased to 40%, the weight of Na_Alg, K_Alg, Mg_Alg, Ca_Alg, chitosan_Alg, and carrageenan_Alg precipitated corresponding to 1/6, 1/6, 1/5.5, 1/5, and 1/5 of their weight. 2/3 weight of Alg mixture (Na_Alg, K_Alg, Mg_Alg, and Ca_Alg) was precipitated in ethanol when ethanol concentration increased to 60% (Table 1). At the condition same, ¼ weight of chitosan_Alg and carrageenan_Alg precipitated in ethanol. The whole weight of the Alg mixture consisting of Na_Alg, K_Alg, Mg_Alg, Ca_Alg, chitosan_Alg, and carrageenan_Alg was precipitated in ethanol more than 80%. The ethanol concentration was proportional to the precipitation of the Alg mixture. The higher the ethanol concentration, the greater the water separation out of the Alg mixture, ionic cross-links in the presence of various divalent cations, e.g. Ca2+, Mg2+, by cross-linking the carboxylic groups of the guluronate groups on the polymer backbone.

Table 1. The precipitation weight of Alg mixture in ethanol for 30 minutes.

| Interaction factor with Alg | Formed mixture | Ethanol (%) |
|----------------------------|----------------|-------------|
|                            |                | 20 | 40 | 60 | 80 | 100 |
| NaCl                       | Na_Alg         | None | 1/6 | 2/3 | All weight |
| KCl                        | K_Alg          | None | 1/6 | 2/3 | All weight |
| CsCl                       | Cs_Alg         | None | 1/5.5 | 2/3 | All weight |
| MgCl                       | Mg_Alg         | None | 1/6 | 2/3 | All weight |
| Chitosan                   | CS_Alg         | PO | 1/5 | 3/4 | All weight |
| Carrageenan                | Ca_Alg         | PO | 1/5 | 3/4 | All weight |

Note: None: Non precipitation; PO: Precipitation appears.

3.2.2. Functional Groups of Alginate in Sargassum duplicatum
The IR spectrum of Alginate extracting from brown alga *Sargassum duplicatum* had the peaks at a wavenumber of 3264 cm⁻¹, 2900 cm⁻¹, 1723 cm⁻¹, 1600 cm⁻¹, 1409 cm⁻¹, 1322 cm⁻¹, 1190 cm⁻¹, and 1027 cm⁻¹ (Figure 1). The peak of 3264 cm⁻¹ showed O=H bonds stretching vibrations that described in the range of 3000–3600 cm⁻¹ in [28] and 3,130 cm⁻¹ in [29]. The peak at 2900 cm⁻¹ in range of 2920–2850 cm⁻¹ and the peak at 1190 cm⁻¹ belonged to the stretching vibrations of aliphatic C=H and the C–O stretching vibration of pyranosyl ring, respectively, described in [28]. The absorption band at 1,612 cm⁻¹ corresponding to the C–C stretch [29], the stretching vibrations of carboxylate anions at around 1610 cm⁻¹ [30] and 1620–1598 cm⁻¹ [28] showed the peaks at wavenumber 1600 cm⁻¹ in the current study can be due to the carboxylate anions stretching vibrations. Reference [31] showed that the peak at 1406 cm⁻¹ related to the deformation vibration of the C=O group and O=C-O symmetric stretching vibration, and in the current study, the IR spectrum of alginic appeared the peak at wavenumber 1409 cm⁻¹. The peak at 1322 was due to the C-N bending vibration, as in 1327 cm⁻¹ in [32]. The peak at 1027 cm⁻¹ may be due to the group of C–C–H and O–C–H, as in the band at 1,089 cm⁻¹ [29] and 1030 cm⁻¹ [31]. According to [28], the ingredients of alginate polymer are sodium homopolysmannuronate that presented at the band of 1100–1010 cm⁻¹. The difference in the signal of functional groups of alginate between the current study and the previous studies can be due to the brown algae species and the extraction method.
3.2.3. Sugar Composition of Alginate in Sargassum duplicatum

Table 2. The precipitation weight of Alg mixture in ethanol for 30 minutes.

| Targets  | Units | Results |
|----------|-------|---------|
| Galactose| mg/g  | 32.00   |
| Rhamnose | mg/g  | 50.15   |
| Manose   | mg/g  | 41.74   |
| Glucose  | mg/g  | 61.41   |

The figure 2 is as follows: Alginate in brown algae Sargassum duplicatum contained different sugar compositions, for example, galactose (32 mg galactose equivalent/g alginate), rhamnose (50.15 mg galactose equivalent/g alginate), mannose (41.74 mg galactose equivalent/g alginate), and glucose (61.41 mg galactose equivalent/g alginate) (Figure 2). Mannuronic acid and guluronic acid are uronic acid derived from mannose and gulose (C-3 epimer of galactose), respectively. Galactose, rhamnose, mannose, and glucose are basic sugar constituents of brown algae joining the formation of alginate.

3.3. Biological Characterization of Alginate

Biological characterization of alginate in the current study focused on the antioxidant activity consisting of total antioxidant activity and reducing power activity. The highest antioxidant activity (35.24±1.14 mg ascorbic acid equivalent/g DW) belonged to chitosan_Alg, compared to other Alg mixtures. The followings were carrageenan_Alg, Mg_Alg, K_Alg, and Na_Alg. The antioxidant activity of Ca_Alg could not analyze because of the too high noise in the analysis and non-disperse Ca_Alg in water.

The insignificant difference in the antioxidant activity of chitosan_Alg and carrageenan_Alg appeared (p>0.05), and was found similar in Mg_Alg, K_Alg, and Na_Alg. The difference was significantly occurring between the Alg salts groups (Mg_Alg, K_Alg, and Na_Alg) and the polymer Alg groups (chitosan_Alg and carrageenan_Alg) (p<0.05).

3.3.1. Total Antioxidant Activity

Total antioxidant activity of various Alg mixtures was 23.62±17.52, 23.54±17.01, 23.66±16.79, 35.24±23.54, and 33.17±21.86 mg ascorbic acid equivalent/g DW, corresponding to Na_Alg, K_Alg, Mg_Alg, chitosan_Alg, and carrageenan_Alg, respectively.
The average total antioxidant activity of polymer Alg groups was 1.45 times of Alg salts (Figure 2). Ca_Alg did exhibit antioxidant activity, suitable for the previous studies. Ca_Alg was useful in the forming of calcium alginate hydrogels, for example, encapsulation of lemon balm antioxidants [33], encapsulation of yerba mate polyphenols [34], and calcium alginate hydrogels consisting of antioxidants, alginates, and chitosan or starch. Chitosan_Alg and carrageenan_Alg exhibited antioxidant activity, suitable for the previous notices on antioxidant activity of alginate [1], carrageenan [35], and chitosan [36].

3.3.2. Reducing Power Activity

Reducing power activity of Alg mixture was in range of 16.79±0.92 and 23.54±1.19 mg FeSO$_4$ equivalent/g DW, corresponding to Mg_Alg and chitosan_Alg, respectively. Reducing power activity of Alg was arranged in the decreasing order as follows: Mg_Alg, K_Alg, Na_Alg, carrageenan_Alg, and chitosan_Alg. The difference between the Alg salts group and polymer Alg group was significant (p<0.05), but did not occur in the group (p>0.05). Reducing power activity of Alg salts varied from 16.79±0.92 to 17.52±1.12 mg FeSO$_4$ equivalent/g DW, corresponding to Mg_Alg and Na_Alg, respectively (Figure 3). Ca_Alg did not possess reducing power activity. The average reducing power activity of polymer Alg mixture was 17.11 mg FeSO$_4$ equivalent/g DW and was 1.33 times of Alg salts mixture. Therefore, metal iron did not play a role in the exhibition on reducing power activity of Alg mixture that depended on the appearance of Alg and bioactive polymer (chitosan and carrageenan).

![Figure 3. Reducing power activity of Alg mixture.](image)

4. Conclusion

Alginate mixture was precipitated for 30 minutes in ethanol concentration by more than 80%. Na_Alg, K_Alg, and Mg_Alg precipitated slower in ethanol, compared to chitosan_Alg and carrageenan_Alg. Alginate mixture possessed antioxidant activity, except for Ca_Alg. The difference in antioxidant activity between a group of Alg salts (Na_Alg, K_Alg, and Mg_Alg) and polymer Alg (Chitosan_Alg and Carrageenan_Alg) happened, but not in the group. The disperse of Na_Alg, K_Alg, and Mg_Alg in the water was the net style. Ca_Alg, chitosan_Alg, and carrageenan_Alg swelled good in the water and existing under a sphere, yarn, and yarn, respectively.

Acknowledgements

The authors thank Nha Trang Institute of Technology Application and Research and Vietnam Academy of Science and Technology for funding.

References

[1] Sellimi S., et al. (2015). Structural, physicochemical and antioxidant properties of sodium alginate isolated from a Tunisian brown seaweed. *Int J Biol Macromol*, 72 1358-1367.
[2] Zaneta K., Krzysztof, M., Dominika, K., Monika, M. and Andrzej, J. (2017). Cytotoxicity, bactericidal, and antioxidant activity of sodium alginate hydrosols treated with direct electric current. *Int J Mol Sci*, 18 678-697.
[3] Milda E. and Kerry, C., Edible films and coatings for food applications, Springer Science+Business Media, 2009, pp. 71.
[4] Martin A. and Cristian, O. (2014). Review of the characterization of sodium alginate by intrinsic viscosity measurements. Comparative analysis between conventional and single point methods. *International Journal of BioMaterials Science and Engineering*, 1 (1): 1-11.
[5] Magdalena B. and Grzegorz, S. (2015). The effect of sodium alginate concentration on the rheological parameters of spinning solutions. *AUTEX Research Journal*, 15 (2): 123-126.
[6] Kuen Y. and David, J. (2012). Alginate: properties and biomedical applications. *Prog Polym Sci*, 37 (1): 106-126.
[7] Kelishomi Z., et al. (2016). Antioxidant activity of low molecular weight alginate produced by thermal treatment. *Food Chem*, 196 897-902.
[8] Mandel K., Daggy, B., Brodie, D. and Jacoby, H. (2000). Review article: alginate-raft formulations in the treatment of heartburn and acid reflux. *Aliment Pharmacol Ther*, 14 669-690.
[9] Leiman D., et al. (2017). Alginate therapy is effective treatment for GERD symptoms: a systematic review and meta-analysis. *Dis Esophagus*, 30 1-9.
[10] Hee M. (2016). Raft formation of sodium alginate in the stomach. *J Neurogastroenterol Motil*, 22 (4): 705-706.
[11] Atsuki Y., Tomokazu, I., Reishi, N. and Ryuichi, N. (2014). Sodium alginate ameliorates indomethacin-induced gastrointestinal mucosal injury via inhibiting translocation in rats. *World J Gastroenterol*, 20 (10): 641-2652.
[12] Aluani D., et al. (2017). Evaluation of biocompatibility and antioxidant efficiency of chitosan-alginate nanoparticles loaded with quercetin. *Int J Biol Macromol*, 103 771-782.
[13] Ahmed M., Shereen, N., Mohie, M. and Mohamed, E. (2016). Effect of microencapsulation on chemical composition and antioxidant activity of cumin and fennel essential oils. *Res J Pharm Biol Chem Sci*, 7 (3): 1565-1574.
[14] Ana B., et al. (2011). Encapsulation of polyphenolic antioxidants from medicinal plant extracts in alginate-chitosan system enhanced with ascorbic acid by electrostatic extrusion. Food Res Int, 44 1094-1101.

[15] Geetha T., Deol, P. and Kaur, I. (2015). Role of sesamol-loaded floating beads in gastric cancers: a pharmacokinetic and biochemical evidence. J Microencapsul, 32 (5): 478-487.

[16] Pilar J., Elena, P., Teresa, S., Javier, O., Fernando, S. and Angel, L. (2005). Free radicals and antioxidant systems in reflux esophagitis and Barrett’s esophagus. World J Gastroenterol, 11 (18): 2697-2703.

[17] Wetscher GJ, Hinder, R., Klingler, P., Gadenstatter, M., Perdikis, G. and Hinder, P. (1997). Reflux esophagitis in humans is a free radical event. Diseases of the Esophagus, 10 29-32.

[18] Norimasa Y. (2007). Inflammation and oxidative stress in gastroesophageal reflux disease. J Clin Biochem Nutr, 40 13-23.

[19] Michael A., Alan, T., Stefan, K., Akira, P., Kjersti, S. and Masahiro, N. (2009). Nineteenth International Seaweed Symposium. J Appl Phycol, 20 (5): 571-577.

[20] Mattio L., Payri, C. and Verlaque, M. (2009). Taxonomic revision and geographic distribution of the subgenus Sargassum (Fucales, Phaeophyceae) in the western and central Pacific islands based on morphological and molecular analyses. J Phycol, 45 (5): 1213-1227.

[21] Ervia Y. and Alim, I. (2016). Characterizing the three different alginate type of Sargassum siliquosum. IJMS, 22 (1): 7-14.

[22] Decky J. I. and Emil, B. (2013). A study of extraction and characterization of alginites obtained from brown macroalgae Sargassum duplicatum and Sargassum crassifolium from Indonesia. 46 (2): 65-70.

[23] Trang S. T. and Pham, T. D. P. (2012). Bioactive compounds from by-products of shrimp processing industry in Vietnam. J Food Drug Anal, 20 (1): 194-197.

[24] Richardson J., Dettmar, P., Hampson, F. and Melia, C. (2004). A simple, high throughput method for the quantification of sodium alginites on oesophageal mucosa. Eur J Pharm Biopharm, 57 299–305.

[25] Prieto P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem, 269 (2): 337-341.

[26] Zhu Q., Hackman, R., Ensunsia, J., Holt, R. and Keen, C. (2002). Antioxidative activities of Oolong tea. J Agric Food Chem, 50 (23): 6929-6934.

[27] A P., Sh, B. and GR, M. (2006). MBA-crosslinked Na-Alg/CMC as a smart full-polyasaccharide superabsorbent hydrogels. Carbohydrate Polymers, 66 (3, 2): 386-395.

[28] Daemi H. and Barikani, M. (2012). Synthesis and characterization of calcium alginate nanoparticles, sodium homopolymannuronate salt and its calcium nanoparticles. Scientia Iranica F, 19 (6): 2023-2028.

[29] Vijayaraghavana G. and Shanthakumar, S. (2016). Performance study on algal alginate as natural coagulant for the removal of Congo red dye. Desalin Water Treat, 57 (14): 6384-6392.

[30] Williams D. and Fleming I. Spectroscopic methods in organic chemistry, 5th, London, 1997, pp. 27-57.

[31] Huisuo H., Ingolf, U. G., Mark, E. and Andrew, D. C. (2017). Measurement of total sodium alginate in restructured fish products using fourier transform infrared spectroscopy. EC Nutrition, 11 (1): 33-45.

[32] Shuting Z. and Jianlong, W. (2018). Modified alginate beads as biosensor and biosorbent for simultaneous detection and removal of cobalt ions from aqueous solution. Environ Prog Sustain, 37 (1): 260-266.

[33] Samira N.-S., Hajar, S. and Mahdi, K. (2016). J Biomater Sci Polym Ed. Encapsulation optimization of lemon balm antioxidants in calcium alginate hydrogels, 27 (16): 1-29.

[34] Alex L. C., Lorena, D. and Miriam Martino (2013). Effect of starch filler on calcium-alginate hydrogels loaded with yerba mate antioxidants. Carbohydr Polym, 95 (1, 5): 315-323.

[35] Yuan H., et al. (2005). Preparation and in vitro antioxidant activity of kappa-carrageenan oligosaccharides and their oversulfated, acetylated, and phosphorylated derivatives. Carbohydr Res, 340 (4): 685-692.

[36] Ngo D. and Kim, S. (2014). Antioxidant effects of chitin, chitosan, and their derivatives. Adv Food Nutr Res, 73 15-31.