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Alginate oligosaccharides: Enzymatic preparation and antioxidant property evaluation

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ABSTRACT

Alginate oligosaccharides (AOs) prepared from alginate, by alginate lyase-mediated depolymerization, were structurally characterized by mass spectrometry, infrared spectrometry and thin layer chromatography. Studies of their antioxidant activities revealed that AOs were able to completely (100%) inhibit lipid oxidation in emulsions, superiorly to ascorbic acid (89% inhibition). AOs showed radical scavenging activity towards ABTS, hydroxyl, and superoxide radicals, which might explain their excellent antioxidant activity. The radical scavenging activity is suggested to originate mainly from the presence of the conjugated alkene acid structure formed during enzymatic depolymerization. According to the resonance hybrid theory, the parent radicals of AOs are delocalized through allylic rearrangement, and as a consequence, the reactive intermediates are stabilized. AOs were weak ferrous ion chelators. This work demonstrated that AOs obtained from a facile enzymatic treatment of abundant alginate is an excellent natural antioxidant, which may find applications in the food industry.

1. Introduction

Compounds with antioxidant activity are attracting considerable attention due to their functions in food preservation and in human health (Shahidi & Zhong, 2010). Currently, both synthetic and natural antioxidants have widespread applications; however, due to the growing trend in consumer preference for natural food ingredients, the interest in using antioxidants from natural sources is increasing (Wanasundara & Shahidi, 2005). This study presents the use of marine alginate as a source of biomaterial in the preparation of natural antioxidants. Alginate is a polysaccharide originating from marine algae, composed of α-L-guluronate and β-D-mannuronate arranged as linear homopolymeric and heteropolymeric blocks (Benvegnu & Sassi, 2010; Pawar & Edgar, 2012). Guluronate and mannuronate are both uronates with carboxylate groups at their C5 positions. The configuration of the carboxylate groups represents the difference between the two (Fig. 1). Approximately 30,000 tons of alginate is produced annually, which is estimated to be less than 10% of the total amount of biosynthesized alginate (Pawar & Edgar, 2012). Hence, alginate can be considered an abundant resource of biomaterials.

During the enzymatic depolymerization of alginate by alginate lyases, the glycosidic linkages are cleaved through *endo*-active β-elimination, and double bonds between the C4 and C5 carbons in the non-reducing terminal residues of the resulting alginate oligosaccharides (AOs) are formed (Fig. 1) (Kim, Lee, & Lee, 2011; Wong, Preston, & Schiller, 2000). Complete depolymerization of alginate by β-eliminating lyases leads to the formation of unsaturated dimers, trimers, and possibly higher oligosaccharides depending on the nature of the lyase (Wong et al., 2000; Zhang et al., 2004).

Zhao et al. (Zhao, Li, Xue, & Sun, 2012) reported that AOs prepared by enzymatic depolymerization of alginate prevent lipid oxidation in emulsions, and they showed that AOs can scavenge hydroxyl (·OH) and superoxide ($O_2^{\cdot-}$) radicals. However, Wang et al. (Wang et al., 2007) reported that AOs have no influence on lipid oxidation in emulsions, and that AOs scavenge only hydroxyl radicals and not superoxide radicals. Trommer and Neubert (2005) reported, that alginic acid has a pro-oxidative effect on lipids in emulsion. Further studies are obviously required to clarify the antioxidant activity of AOs and the molecular basis for this activity. This study aims to clarify the molecular mechanism of the

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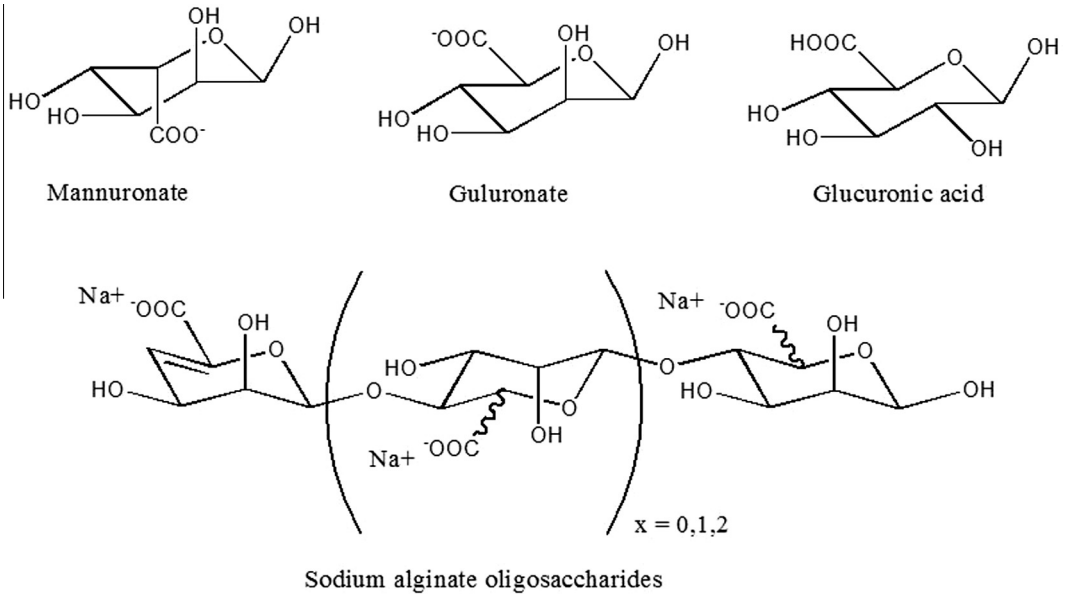

Fig. 1. Saccharide structures.

antioxidant activity of AOs through comparative studies with other polymeric and monomeric forms of alginate.

Several antioxidant mechanisms have been proposed generally for carbohydrates, including the ability to scavenge reactive oxygen species such as superoxide radicals and hydroxyl radicals. Three main mechanisms exist by which compounds can directly scavenge free radicals, namely single electron transfer (SET), hydrogen atom transfer (HAT), and radical addition to e.g. double bonds (Hernandez-Marin & Martinez, 2012). Through their theoretical studies of the radical scavenging capability of carbohydrates, Hernandez-Marin et al. (Hernandez-Marin & Martinez, 2012) concluded that SET is less likely to occur, and that HAT mainly occurs from carbon-bonded hydrogens. Radical addition is generally not considered a possible antioxidant mechanism of carbohydrates, as they do not commonly contain double bonds or aromatic rings. In their study of a range of antioxidants, Peshev et al. (Peshev, Vergauwen, Moglia, Hideg, & Van den Ende, 2013) observed that compounds with a carbon–carbon double bond were superior antioxidants. The double bond provides an opportunity for radical addition, which becomes the preferred radical reaction over SET and HAT. As enzymatically prepared AOs have double bonds in their structure, we hypothesized that AOs are endowed with increased antioxidant activity in terms of increased radical scavenging capability through both hydrogen abstraction and radical addition.

The present study aimed to investigate the antioxidant properties of AOs prepared by complete depolymerization of sodium alginate by a β-eliminating lyase. The release of unsaturated saccharides during depolymerization was followed by spectrophotometric analyses. The AOs were recovered when the lowest possible degree of polymerization of alginate was obtained, i.e. when additional enzymatic treatment did not lead to additional depolymerization. The composition and antioxidant properties (lipid oxidation inhibition, radical scavenging activity ($^{\cdot}OH$, $O_2^{\cdot-}$, and $ABTS^{\cdot}$), and ferrous ion chelating activity) of the AOs were investigated. Comparative studies were made on polymeric, oligomeric, and monomeric forms of alginate; on mannuronate- and guluronate-rich fractions; and on acid and salt forms of the AOs, in order to determine which functional group(s) of AOs are responsible for their antioxidant activity.

2. Materials and methods

2.1. Materials

Sodium alginate (Grindsted® Alginate FD 170) was provided by DuPont, previously Danisco, Brabrand, Denmark. This alginate originated from brown algae, and the ratio of α-L-guluronate units to β-D-mannuronate units was 40–60. Alginate lyase S from *Sphingobacterium* was provided by Nagase Enzymes, Kyoto, Japan. Amberlite® 200 Na^+ strong cation exchanger resin, Tween® 20, ammonium acetate, phosphate buffered saline (PBS) 0.01 M pH 7.4, D-glucuronic acid, ascorbic acid, ferrous sulfate ($FeSO_4$), trichloroacetic acid, thiobarbituric acid (TBA), potassium hydroxide (KOH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate ($K_2S_2O_8$), sodium salicylate, hydrogen peroxide (H_2O_2), xanthine oxidase (from bovine milk, 0.11 U/mg solid, XOD), hypoxanthine (minimum 99%, HPX), nitrotetrazolium blue chloride (NBT), ferrous chloride ($FeCl_2$), 3-(2-pyridyl)-5, 6-diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt (ferrozine), and disodium ethylenediaminetetraacetate dehydrate (EDTA–$Na_2{\cdot}2H_2O$), were purchased from Sigma–Aldrich. Linoleic acid 96% was obtained from Zhongchuan Biotechnology Co Ltd. Anqing, China.

2.2. Enzymatic depolymerization of sodium alginate and purification of AOs

Sodium alginate (3.0 g) was depolymerized in 150 ml 0.05 M ammonium acetate using alginate lyase S at a concentration of 5% w/w of sodium alginate, at 35 °C and 200 rpm. Aliquots of 200 μl of the reaction mixture were withdrawn at time: 0, 0.5, 1, 2, 4, 8, 12, 24, 27, 30, 33, 48, 51, 53, and 72 h. The lyase in the samples was denatured at 110 °C for 15 min and removed by centrifugation for 5 min at 10,000 rpm at room temperature. The release of unsaturated saccharides was determined by measuring the absorbance of the supernatant at 234 nm in a UV–visible spectrophotometer (Cary 50Bio, Varian, Australia) using a quartz cuvette. The supernatant was diluted in pure water to obtain a value of absorbance within the accurate range of the spectrophotometer. The course of depolymerization was additionally followed