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Effects of sulfated polysaccharides from green alga *Ulva intestinalis* on physicochemical properties and microstructure of silver carp surimi

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ABSTRACT

The aim of this study was to isolate sulfated polysaccharides from green alga *Ulva intestinalis* (UIP) and evaluate its potential application as a new hydrocolloid in surimi formulation. Compositional analysis showed that UIP comprised carbohydrate (65.3%), protein (8.4%), sulfate (18%) and uronic acid (5.6%) with the average molecular weight of 64.2×10^3 g mol^{-1}. FT-IR revealed the presence of sulfate esters as indicated by the peaks at 826 (C–O–S) and 1257 (S–O) cm^{-1}. In order to examine the effects of UIP on the characteristics of silver carp surimi, it was added into surimi paste in four levels (0.25–1.0 g/100 g) while protein/water contents were kept constant. The UIP addition reduced the pH of surimi gel from 6.6 to 6.4. Emulsifying stability of surimi pastes were notably improved by UIP addition while emulsification capacity showed no changes. The addition of UIP up to 0.25 g/100 g maintained the textural characteristics and water holding capacity of surimi gels. The gel whiteness was not adversely changed by UIP addition up to 0.75 g/100 g. The SEM images revealed the presence of compact microstructures with dense surfaces due to UIP addition. All products formulated with different UIP levels were judged acceptable by the sensory panel. Based on the present study, the incorporation of seaweed sulfated polysaccharides at low concentrations into surimi are not likely to compromise quality and therefore could be suggested as a functional ingredient in surimi and surimi-based products with beneficial health effects.

1. Introduction

Hydrocolloids are a diverse group of long-chain polymers with an incredibly high affinity for water. Hydrocolloids include naturally occurring polysaccharides with interesting rheological properties; however protein gelatin is an exceptional member which has become accepted in this group due to its excellent hydrophilicity and polydispersity (Dickinson, 2003). Among seaweed originated polysaccharides used as food hydrocolloids, there has been a particular attention toward their sulfated form in recent years, mainly due to their diverse textural features and therapeutic potentials (Chapman & Baek, 2002).

Sulfated polysaccharides from green seaweeds have shown promising biological activities such as antitumor (Kim, Cho, Karnjanapratum, Shin, & You, 2014), immunomodulating (Tabarsa, Han, Kim, & You, 2012), antihyperlipidemic (Qi,Liu, et al., 2012), antioxidant (Godard, Décordé, Ventura, Soteras, Baccou, Cristol, & Rouanet, 2009) and anticoagulant activities (Qi, Mao et al., 2012). Besides, previous studies reported that sulfated polysaccharides possess a number of physicochemical properties such as high water binding capacity which makes them ideal to be utilized in pharmaceutical, cosmetics and food industries (Reviers & Leproux., 1993; Robic, Gaillard, Sassi, Lerat, & Lahaye, 2009; Yaich, Garna, Besbes, Barthélemy, Paquot, Blecker, and Attia, 2014). Polar groups of the polysaccharides including hydroxyl, carboxyl and sulfate groups form hydrogen bonding with water to maintain moisture. In addition, the polysaccharide chains may generate a network which plays an important role in ensuring moisture retention (Shao, Shao, Han, Lv, & Sun, 2015). Although, previous studies reported surfactant property of green seaweed polysaccharides and its probable utilization in food industry as a novel emulsifying hydrocolloid, there was no investigation on direct addition of these polysaccharides to a food product in order to evaluate its hydrocolloidal effects in a real food system (Shao, Qin,

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Han, & Sun, 2014; Shao, Shao, Jiang, & Sun, 2016; Tian, Yin, Zeng, Zhu, & Chen, 2015).

Surimi, a fish myofibrillar protein concentrate, is an intermediate foodstuff to produce various texturized products. Fish surimi products have been widely accepted by the public because of their high nutritional quality and pleasant taste. Moreover, surimi technology is an excellent way to increase fish consumption, particularly in countries like Iran, with annual per capita fish consumption of 10 kg as against the global per capita fish consumption of more than 20 kg/year. Surimi products also provide the chance for optimal use of less popular and underutilized fish species as human food (Salehi, 2016).

Silver carp (*Hypophthalmichthys molitrix*), a warm water fish farmed extensively in Iran, is a good candidate for surimi-based products due to its rapid growth, high yield, low price as well as the white color which makes it suitable for surimi production (Shaviklo & Fahim, 2014). Moreover, nowadays the relationship between diet and health is more understood by consumers which together with an increase in the competitive environments of industries have led food specialists to put more emphasis on the development of new products with functional ingredients, than ever. In this regard, several studies have been conducted on the impact of functional ingredients addition as hydrocolloid in the surimi formulation such as konjac glucomannan, oat bran, chicory root inulin, carrageenan and xanthan gum in order to meet both nutritional and mechanical properties of surimi gel (Alakhrash, Anyanwu, & Tahergorabi, 2016; Cardoso, Mendes, & Nunes, 2007; Eom et al., 2013; Kim, Park, & Choi, 2003; Ramirez, Barrera, Morales, & Vazquez, 2002; Xiong et al., 2009).

In the view of increasing attention of food specialists and technologists to hydrocolloids with specific functionalities, introducing a new polysaccharide with both functional and biological characteristics will be invaluable (Yadav, Johnston, Hotchkiss, & Hicks, 2007). The utilization of these hydrocolloids in fishery restructured products will also address the needs of consumers to dietary fiber intakes (Cofrades, Lopez-Lopez, Solas, Bravo, & Jimenez-Colmenero, 2008).

Therefore, in current study, we characterized the structural and molecular properties of sulfated polysaccharides from *Ulva intestinalis* and aimed at the evaluation of textural, color, microstructure and sensory properties of silver carp surimi upon the addition of sulfated polysaccharide.

2. Materials and methods

2.1. Materials

The green seaweed *U. intestinalis* was collected from the coast of Noor, Mazandaran province, Iran in July 2015. The seaweed was thoroughly washed with sea water, followed by tap water and then oven-dried at 60 °C. The dried raw materials were milled using a blender, sieved (<0.4 mm) and stored in a sealed plastic bag at −20 °C until using.

Frozen and vacuum-packed silver carp surimi blocks (1 kg each) were purchased from the national fish processing research center (NFPRC, Anzali, Guilan, Iran), and transported in boxes filled with ice to the laboratory. Upon arrival, surimi block was cut into approximately 500 g units, and stored at −80 °C until needed. The storage time of surimi did not exceed three months. Surimi contained cryoprotectants including sodium tripolyphosphate (0.3 g/100 g), sucrose (4 g/100 g) and sorbitol (4 g/100 g). The moisture content of surimi was determined as 77.1 g/100 g (AOAC, 2000). All of the chemicals and reagents used in this study were analytical grade.

2.2. Sulfated polysaccharide preparation

The milled sample (20 g) was treated with 80% ethanol (EtOH, 200 mL) under constant mechanical stirring overnight at room temperature to remove lipids, free sugars, amino acids and some phenols. The residual part was separated by centrifugation (10 °C, 6080 g, 10 min), rewashed with EtOH, rinsed with acetone, centrifuged at 10 °C and 6080 g for 10 min again and dried at room temperature in a fume hood. To extract the polysaccharides, 30 g of depigmented sample was extracted with distilled water (600 mL) at 65 °C with stirring for 2 h. The extracts were centrifuged at 9500 g for 10 min at 10 °C. The extraction was conducted twice. The supernatants were concentrated by rotary at 60 °C to approximately 200 mL. Cold EtOH (99%) was added into the supernatants to obtain the final concentration of 70%, and then stored overnight at 4 °C. The precipitate was obtained by centrifugation at 7700g for 10 min at 18 °C. The pellet was washed with EtOH (99%) and acetone, and then dried at room temperature in a fume hood. The precipitated polysaccharide was designated as UIP and the yield was calculated according to the dried biomass obtained after treating the milled sample with 80% EtOH.

2.3. Surimi gel preparation

Surimi was cut into small pieces after thawing in a refrigerator (4 °C) and then was chopped for 1 min in a food processor (FP270, Kenwood, Hong Kong, China). Salt (2 g/100 g) was added into surimi and chopped for 0.5 min. Then, chilled water (4 °C) was added to adjust the final moisture content to 78 g/100 g. In order to maintain the same protein concentration and moisture content for all treatment groups, SiO_2 (silicon dioxide, Merck, Darmstadt, Germany) was added to surimi paste as inert filler (Tahergorabi, Beamer, Matak, & Jaczynski, 2012). Polysaccharide (ground in a mill and passed through a sieve with a mesh of 140) and SiO2 were added to the surimi paste in four combinations along with one treatment as a control (Table 1). Mixing was continued for another 4 min to obtain a homogenate paste. The pastes were then stuffed into plastic casing (25 mm in diameter and 150 mm in length), both ends were sealed tightly and were heated in a water bath at 90 °C for 30 min. The gels were then submerged in iced water for 30 min before storing overnight in a refrigerator (4 °C) for further analysis.

2.4. Analytical methods

2.4.1. Chemical composition of UIP

Sulfate content of the polysaccharide was determined by barium chloride–gelatin method (Dodgson & Price, 1962). Uronic acid content was determined by sulfamate/m-hydroxydiphenyl assay using glucuronic acid as standard (Filisetti-Cozzi & Carpita, 1991). Total carbohydrate content was obtained by a phenol– sulfuric acid assay using D-glucose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein content was estimated using Lowry method (Lowry, Rosenbrough, Farr, & Randall, 1951). Ash content was determined according to the method of Lahaye and Jegou (1993).

2.4.2. Average molecular weight determination of UIP

The sample molecules of 4 mg were dissolved in distilled water (2 mL) followed by heating in a microwave bomb (#4872; Parr Instrument Co., Moline, IL, USA) for 30 s. The heated sample solution was filtered through a cellulose acetate membrane (3.0 μm-pore size; Whatman International) before injection into the high performance size exclusion chromatography column, which was linked to a UV, multi-angle laser light scattering and refractive index detection (HPSEC-UV-MALLS-RI) system. The HPSEC-UV-