Colloids and Surfaces B: Biointerfaces 113 (2014) 107–114

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Stabilization of water in oil in water (W/O/W) emulsion using whey protein isolate-conjugated durian seed gum: Enhancement of interfacial activity through conjugation process

Bahareh Tabatabaee Amid, Hamed Mirhosseini*

Department of Food Technology, Faculty of Food Science and Technology, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 27 April 2013
Received in revised form 4 August 2013
Accepted 26 August 2013
Available online 1 September 2013

Keywords:
Conjugation process
Durian seed gum
Whey protein isolate
Multiple W/O/W emulsion
Maillard reaction
Interfacial emulsifying activity

ABSTRACT

The present work was conducted to investigate the effect of purification and conjugation processes on functional properties of durian seed gum (DSG) used for stabilization of water in oil in water (W/O/W) emulsion. Whey protein isolate (WPI) was conjugated to durian seed gum through the covalent linkage. In order to prepare WPI–DSG conjugate, covalent linkage of whey protein isolate to durian seed gum was obtained by Maillard reaction induced by heating at 60 °C and 80% (±1%) relative humidity. SDS-polyacrylamide gel electrophoresis was used to test the formation of the covalent linkage between whey protein isolate and durian seed gum after conjugation process. In this study, W/O/W stabilized by WPI-conjugated DSG A showed the highest interface activity and lowest creaming layer among all prepared emulsions. This indicated that the partial conjugation of WPI to DSG significantly improved its functional characteristics in W/O/W emulsion. The addition of WPI-conjugated DSG to W/O/W emulsion increased the viscosity more than non-conjugated durian seed gum (or control). This might be due to possible increment of the molecular weight after linking the protein fraction to the structure of durian seed gum through the conjugation process.

1. Introduction

Durian seed gum (DSG) is a heteropolysaccharide-protein complex from *Durio zibethinus* seed. This complex polysaccharide gum is composed of galactose and glucose as major monosaccharide as well as arabinose and xylose as minor monosaccharide [1]. DSG showed a potential emulsifying activity in oil in water (O/W) emulsion as reported previously [2]. The emulsifying activity could be due to the presence of a low amount of the protein fraction in the chemical structure of DSG even after purification process. However, there is a possibility to improve the functional properties of DSG through different processes (such as protein conjugation).

Both polysaccharides and proteins play a crucial role in stabilizing the emulsion system through steric and electrostatic stabilization. In this regard, the linkage of protein to the polysaccharide structure can promote its functional properties in the bulk solution and dispersion system [3]. In fact, protein/polysaccharide (PP) conjugate shows much better interfacial emulsifying activity than the single form of protein or polysaccharide in the emulsion system. However, the interfacial activity of polysaccharide-protein conjugate is influenced by the type and content of protein fraction attached to the gum structure. The formation of protein–polysaccharide conjugate is based on a dry-heating reaction without any chemical catalyst at controlled humidity via Maillard-type conjugation [4]. This will result in the formation of covalent linkage between protein and polysaccharide (Fig. 1). During the conjugation process, the formation of non-covalent electrostatic interactions between protein and polysaccharide can also result in the new hybrid polymer [5]. However, the formation of the covalent linkage and/or non-covalent electrostatic interactions between protein and polysaccharide depends on the stage and condition of Maillard reaction. In the early stage, Maillard reaction may induce the non-covalent electrostatic interactions between protein and polysaccharide and consequently form a new hybrid polymer with relatively darker color than its original form. On the other hand, the advanced Maillard reaction can accelerate the formation of covalent linkage. This will result in the formation of less soluble polymer with much darker color than its original color. In most cases, the conjugated protein–polysaccharide polymer has significantly larger molecular weight polymer and better emulsifying activity than protein or polysaccharide alone.

Water in oil in water (W/O/W) emulsion is considered as a multiple layer emulsion. In this emulsion, small water droplets are initially dispersed into large oil droplets to prepare the primary W/O emulsion phase. Double emulsions offer more advantages

* Corresponding author. Tel.: +60 389468390; fax: +60 389423552.
E-mail address: hamedmi@food.upm.edu.my (H. Mirhosseini).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.08.042

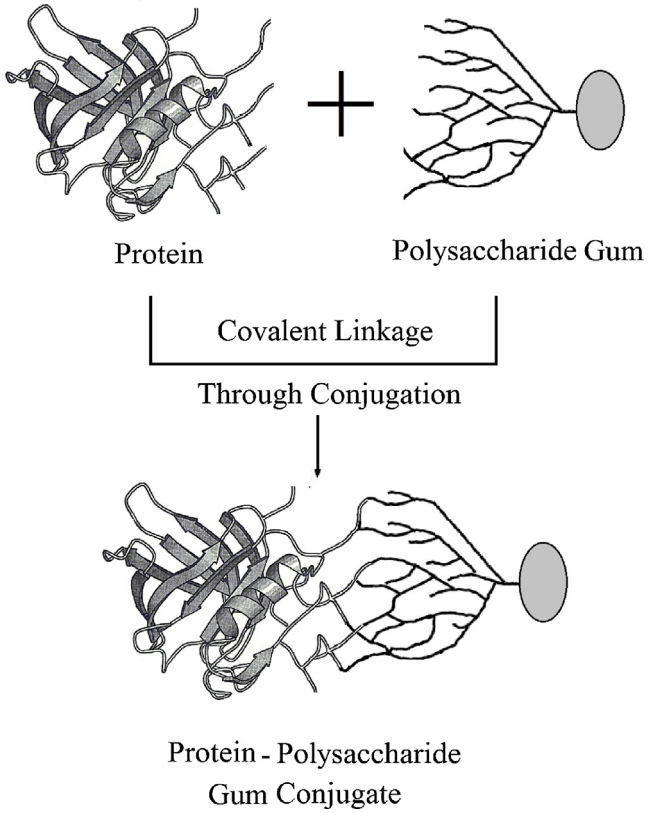

Fig. 1. Schematic image of the covalent linkage between protein and polysaccharide gum through conjugation.

than simple emulsions due to their ability to carry both polar and non-polar cargos. They exhibit more efficient controlled release than the single emulsion [6]. However, it depends on functional properties of target emulsifiers (both low and high HLB surfactants) and other emulsion components in W/O/W emulsion.

The main objective of the present study was to investigate the effect of purification and conjugation process on functional properties of durian seed gum (DSG) in water in oil in water (W/O/W) emulsion. In this study, whey protein isolate (WPI) was conjugated to durian seed gum (DSG) through Maillard reaction induced by the conjugation process. It was hypothesized that the interfacial emulsifying activity of DSG could be enhanced by the covalent linkage with WPI through the conjugation process. The emulsifying function of differently purified and conjugated DSGs was determined by assessing the average droplet size, polydispersity index (PDI), specific surface area, viscosity and creaming stability of different multiple emulsions. To the best our knowledge, there is no published report studying the effect of conjugation process on emulsifying activity of DSG in the multiple W/O/W emulsion.

2. Materials and methods

2.1. Materials

Isopropanol, ethanol (95% and 99.9%), acetic acid, acetone, Fehling solution, glycerol, hydrochloric acid, saturated barium hydroxide, sodium azide and Tween 80 were purchased from Fisher Scientific (Pittsburgh, PA, USA). Whey protein isolate (Proven A 190) was provided by Glanbia Nutritionals (Monreo, WI, USA). Polyglycerol polyricinoleate (PGPR 4150) was supplied by Palsgaard Ltd. (Palsgaardvej 10, Juelsminde, Denmark). Durian (*D. zibethinus*) fruits and soybean oil were purchased from the local market and supermarket (Selongor, Malaysia).

2.2. Pretreatment of durian seed followed by further gum extraction

Durian fruits were de-husked and removed the seed. The seeds were cleaned and rinsed thoroughly with sterile distilled water and chopped into small pieces. Then, it was air dried at 25 °C by using the air circulation before milling into flour and passing through a 500 μm sieve. It was defatted by means of extract the oil from the durian seed flour in order to avoid the thermal degradation. The defatting process was carried out by using hexane and isopropanol (60:40) as previously reported [7]. The solvent residue was removed by centrifugation at 1400 × *g* for 15 min (Avanti J-25 Centrifuge, Beckman Coulter GmbH, Krefeld, Germany). Then, defatted-durian seed flour (1 kg) was exhaustively discolored using ethanol.

The chemical extraction was performed according to the method described by Nwokocha and Williams [8] with the minor modification. 5 g of defatted and disolored durian seed gum (DSG) was dispersed in 400 ml deionized water and stirred for 6 h by a magnetic stirrer. Then, it was centrifuged at 7560 × *g* for 30 min. The residue was reconstituted repeatedly with deionized water, stirred and centrifuged again. The supernatant was treated with 2-propanol when the gum spooled out. The solvent residue was decanted by filtering under suction in a Buchner funnel. The crude gum was collected and oven dried at 40 °C overnight and stored in an airtight container. The gum preparation was carried out in triplicate.

2.3. Purification of durian seed gum (DSG)

In the previous study [9], the effect of four different purification methods ((i) ethanol and isopropanol, (ii) isopropanol and acetone, (iii) saturated barium hydroxide and (iv) Fehling solution) on physicochemical and functional properties of durian seed gum was examined. The purification using saturated barium hydroxide and Fehling solution provided the purified DSG with more desirable solubility and water holding capacity (WHC), better yield and smaller particle size and uniformity than two other purification methods [9]. Therefore, these purification methods (using saturated barium hydroxide and Fehling solution) were chosen as the most efficient methods for the removal of undesirable impurities from DSG in the current work. In this study, the purification using saturated barium hydroxide (method A) was applied according to the method described by Singh et al. [10]. In this method, the gum solution (2.5%, w/v) was prepared by dissolving 2.5 g of the crude DSG in 100 ml of water and continuous stirring for 12 h at 60 °C. Then, the gum solution was precipitated with saturated barium hydroxide solution. The precipitate was separated by a Beckman centrifuge (Avanti J-25 Centrifuge, Fullerton, CA, USA) at 15,180 × *g* for 15 min. Then, the precipitate was stirred with 1 M acetic acid for 8 h and again centrifuged at 15,180 × *g* for 15 min. The supernatant was precipitated with 90% ethanol and then the precipitate was washed with 95% ethanol and oven dried at 40 °C.

In the current study, the purification using Fehling solution (method B) was employed according to the method described earlier [9]. Initially, 1 g of the crude DSG was dissolved in approximately 100 ml of water and stirred for 24 h with magnetic stirring. The prepared gum solution (1%, w/v) was precipitated by adding 5 ml of freshly prepared Fehling solution and the precipitate was collected by the glass filter (No. 3). Then, the precipitate was dissolved in 0.1 M hydrochloric acid by a magnetic stirrer for 1 h until the full solubilization. The solution was precipitated with three volumes of 95% ethanol. The precipitate was separated by the glass