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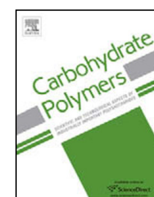

Improvement of emulsifying properties of oat protein isolate–dextran conjugates by glycation

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ABSTRACT

In order to improve the emulsifying properties of oat protein, oat protein isolate (OPI)–dextran (Dex) conjugates were prepared by glycation reaction. Emulsifying properties of emulsions stabilized by native OPI (OPI_N), OPI–Dex conjugates (ODC) and heated OPI (OPI_H) were characterized by zeta-potential, mean droplet size and microstructure. The results showed that the covalent attachment of OPI and dextran was confirmed by determining degree of graft and SDS–PAGE. OPI–Dex conjugates were capable of forming a finer emulsion, which exhibited smaller average particle size and better storage stability under different homogenization pressures (30, 60, 90 MPa) compared with OPI_N and OPI_H. When assessed in different pH and ionic strength, emulsions stabilized by OPI–Dex conjugates resulted in improved emulsion stability to environmental stresses. Confocal laser scanning microscopy depicted more uniform and smaller oil droplets that had a reduced tendency to coalesce for emulsions prepared with ODC.

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1. Introduction

Finely dispersed oil-in-water (O/W) emulsions play an important role in food, nutrition, and pharmaceutical industries, particularly for food formulations such as butter, milk and ice cream. Emulsion is a complex and thermodynamically unstable system, produced by the dispersion of two mutually immiscible liquids into one another through mixing, shearing, and homogenization (McClements, 2005). High-pressure homogenizers are particularly suitable for producing finely dispersed emulsions in the dairy industry (Desrumaux & Marcand, 2002). In emulsified food products the mean diameter of the droplets typically range from 0.1 to 100 μm. With amphiphilic characteristics, proteins are commonly used as emulsifiers in the stabilization of emulsions in the food industry. Once adsorbed onto the droplet surface of emulsions, proteins may undergo changes both in conformation and interactions due to the new environment, and act in two ways: lowering interfacial tension and forming a protective coating that prevents the droplets from aggregating (Wilde, Mackie, Husband, Gunning, & Morris, 2004). The properties of interfacial layer are governed by the structure and composition of the adsorbed protein and in turn would influence the emulsifying properties of an O/W emulsion (Rodríguez Patino & Pilosof, 2011). However, oil-in-water (O/W) emulsion is tend to break down for a variety of physicochemical mechanisms. Emulsions stabilized by protein are highly susceptible to environmental stresses e.g. pH, ionic strength, and temperature changes, and finally result in aggregation if the pH is close to the isoelectric point of the protein and/or in high ionic strength (Kim, Decker, & McClements, 2002). A reasonable explain is electrostatic screening of the charges lead to decrease the electrostatic repulsion between the emulsion droplets and creaming and coalescence occurs (Dickinson, 2008). The improvement of the emulsifying properties of protein is beneficial to broaden its utilization in the production of emulsion-based foods with excellent desired functionalities.

Recently a further area that has attracted considerable attention is the use of proteins derived from cereal crops due to the increased health-related demand of protein ingredients with enhanced functionality. As a characteristic coarse cereal, oats are rich in valuable nutrients such as proteins, dietary fibers, vitamins, unsaturated fatty acids and phytochemicals (Konak et al., 2014). Oats possess the highest protein level among cereals, typically ranging from 12% to 20% (Mohamed, Biresaw, Xu, Hojilla-Evangelista, & Rayas-Duarte, 2009). With a chemical score of 72–75, oat protein isolate shows better nutritive quality due to relatively high content of limiting amino acids lysine (Klose & Arendt, 2012). In oats globulins are the predominant storage proteins and comprise over 50% of the total proteins. They are salt soluble proteins that tolerate high

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temperatures. Oat globulin is an oligomeric protein with six quaternary monomer 54–60 kDa subunits which resembles the structure of soy 11S globulin (glycinin) (Chang, Alli, Konishi, & Ziomek, 2011).

Proteins prepared from oats, attracting more and more attention, are superior sources of plant proteins, but are not used extensively for human consumption. In order to make full use of oat protein as a food ingredient, it would be desirable to improve its functionality. Previous research indicated that oat protein isolate showed poor functional properties at slightly acidic and neutral pH levels (Ma, 1983, 1985). Some physical and chemical modification methods, such as enzymatic hydrolysis (Guan, Yao, Chen, Shan, & Zhang, 2007), succinylation and deamidation (Laine et al., 2011; Mirmoghtadaie, Kadivar, & Shahedi, 2009) of oat protein, have been used to improve emulsifying property, solubility, foaming and gelling property to a certain extent. However, few of these modification is suitable for food application due to various food safety problems. One promising approach is using protein–polysaccharide conjugates via a Maillard reaction and the Maillard reaction occurs under mild and safe conditions without extraneous chemicals. Maillard (1912) first reported the Maillard reaction, a complex form of non-enzymatic browning, which involves a chemical reaction between an available amino group on a protein and a reducing end of a polysaccharide. Glycation is based on the Amadori rearrangement steps of the Maillard reaction (Maillard, 1912). In fact, both wet and dry reaction system have been reported. Dry-heating system requires a controlled environment and reacts for long time up to several days or weeks, which is not feasible in food industrial production. While in aqueous system, it has the advantage of accelerating the reaction rate and acceptable browning color. It has been successfully applied in whey protein (Zhu, Damodaran, & Lucey, 2010), wheat germ protein (Niu, Jiang, Pan, & Zhai, 2011) and soy protein (Qi, Liao, Yin, Zhu, & Yang, 2010) to improve their emulsifying properties. Protein–polysaccharide conjugates can greatly improve the functional properties, including emulsifying properties, solubility particularly around the isoelectric pH and heat stability (Liu, Ru, & Ding, 2012). The enhanced emulsifying properties result in reducing oil droplet size and increasing emulsion stabilization against creaming (McClements, 2007). The reason for this excellent improvement is attributed to hydrophobic protein absorbed to the surface of the oil droplet, while the bound hydrophilic polysaccharides could enhance steric stabilization forces between oil droplets (Oliver, Stanley, & Melton, 2006). Droplets coated by thick interfacial membranes also have improved stability to environmental stresses than those stabilized by single layered membranes.

Therefore, glycation is a promising way for protein modification to improve their functional properties. However, limited information is available concerning about the effects of glycation reaction on the emulsifying properties of oat protein. The objective of this research was to evaluate the emulsifying properties of OPI–dextran conjugates produced by glycation reaction in an aqueous solution. The emulsions were prepared under different homogenization pressures, pH and ionic strength. The oil droplet size, zeta-potential and microstructure were also examined.

2. Materials and methods

2.1. Extraction of oat protein isolate (OPI)

Oats purchased from a local supermarket were milled with passing 80 mesh and then defatted with *n*-hexane. Defatted oat flour was dispersed in deionized water (1:10, w/v) and the pH was adjusted to 9.5 using 1 mol/L NaOH. The dispersion was gently stirred for 2 h to extract protein and then centrifuged at 3170 × *g* for 15 min. The supernatant was adjusted to pH 4.5 with 1 mol/L HCl and centrifuged at 3170 × *g* for 15 min. The isoelectrically precipitated protein was washed twice by deionized water and then neutralized to pH 7.0 before lyophilization (Liu et al., 2009). Protein content in OPI powder was 90.0 g/100 g as determined by Kjeldahl method ($N \times 5.83$).

2.2. Preparation of OPI–dextran conjugates

The conjugating of OPI and dextran40 (M_w 40 kDa) was carried out according to a method of Zhu (Zhu et al., 2010). Lyophilized OPI (2%, w/w) was suspended in 20 mmol/L PBS at pH 9.0 and stirred for 2 h at room temperature to a uniform dispersion. Sodium azide (0.02%, w/w) was added as an anti-microbiological agent. Dex40 (2%, w/w) were added to the dispersion and the mixtures were stirred for another 2 h for the complete hydration and then stored at 4 °C overnight. Aliquots of the solutions were then transferred to 20 mL screw-capped tubes and incubated in a water bath at 90 °C. The reacted tube was removed at 0, 20, 40, 60, 80 and 100 min, respectively and cooled in an ice bath and then centrifuged. The supernatant termed as OPI–dextran conjugates (ODC) was collected and stored at 4 °C until further testing. As control, the native OPI solution was named OPI_N and the OPI heated without Dex40 in a similar manner (heated control) was referred to as OPI_H. The unheated mixture of OPI and Dex 40 kDa was defined as mixture.

2.3. Determination of extent of glycation

The degree of glycation (DG) was determined by the changes of free amino groups, using the TNBS method described by Snyder with slight modifications (Snyder & Sobocinski, 1975). In brief, 200 μL of the diluted solutions were mixed with 2.0 mL of 1 mg/mL SDS in PBS (0.2 mol/L, pH 8.0) and 1.0 mL of 0.1 mg/mL 2,4,6-trinitrobenzene sulfonic acid (TNBS) in aluminum-covered test tubes, followed by incubation in the dark for 1 h at 50 °C. The reaction was terminated by adding 2.0 mL of 0.1 mol/L sodium sulfite. The mixture was cooled at room temperature for 15 min, the absorbance was measured at 420 nm. The control was prepared in same manner except that distilled water was used instead of samples. The free amine content was calculated from the standard curve of L-leucine and the DG was calculated as follows:

$$DG = \frac{C_0 - C_t}{C_0} \times 100\%$$

where C_0 (mmol/mg protein) is the free amino groups (mainly ε-amino) content of OPI_N and C_t is the free amine content of the congujates after reacting for *t* (min).

2.4. SDS–PAGE

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was conducted according to the method of Laemmli using 5% (v/v) stacking gel and 12% (v/v) separating gel, respectively (Laemmli, 1970). Sample solution (20 μL at 2 mg/mL protein) was loaded into each well. The electrophoresis was conducted at a constant voltage of 100 V. After electrophoresis, one gel was stained for protein by Coomassie blue R250 and then the other gel was stained for carbohydrate by Schiff's reagent.

2.5. Emulsion preparation

Oil-in-water (O/W) emulsions were prepared by mixing 95% (v/v) protein solution (5 mg/mL) with 5% (v/v) commercial soybean oil using a homogenizer Ultra Turrax model (Ika T18 Basic,

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