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Emulsifying properties of vicilins: Dependence on the protein type and concentration

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ABSTRACT

The emulsifying and interfacial properties of vicilins from three selected *Phaseolus* legumes (kidney, red and mung beans; denoted as KV, RV and MV, respectively) at protein concentrations (c) of 0.25–2.5% (w/v) were investigated. The results indicated that the emulsifying ability and emulsion stability (against flocculation, coalescence and even creaming) of vicilins considerably varied with their type and c. In general, KV exhibited much better emulsifying ability than RV or MV. Increasing c basically facilitated formation of the fresh emulsions with less flocculated oil droplets, and enhanced their stability against coalescence. Increasing c progressively decreased the stability of KV and RV emulsions against flocculation and creaming, while in the MV case, least stability was observed at $c = 1.0\%$. Besides the differences in conformational characteristics, the differences in emulsifying properties of these vicilins could be to a certain extent attributed to the differences in their interfacial properties, including ζ-potential and interfacial protein concentration, as well as unfolding, penetration and structural rearrangement at the interface. The findings are of great importance for extending the current knowledge about the emulsifying properties of vicilins, thus providing valuable information for the development of vegetable protein-stabilized emulsion products.

1. Introduction

Seed storage proteins become more nutritionally and functionally valuable in the food industry. The major storage proteins are globulins and albumin, with the former further subdivided into 7S or vicilins and 11S or legumins (Tandan-Silvas, Tecson-Mendoza, Mikami, Utsumi, & Maruyama, 2011). In general, legumes contain mostly globulins, with total globulin content and relative vicilin/legumin ratios varying considerably with type and species of legumes (Boye, Zare, & Pletch, 2010). *Phaseolus* legumes (*Phaseolus* L.) have been cultivated for several thousand years all over the world, especially in America but also in Europe, Asia and Africa (Sathe, 2002). The exact protein content in *Phaseolus* beans varies in the range 20–30% on a dry weight basis. The major storage proteins of these beans are vicilin and legumin, with vicilin as the prominent component. Vicilin is a 7S or 8S globulin composed of 3–5 subunits and represents about 50% of total protein content (Makri & Doxastakis, 2006). In the recent decades, there are increasing interests in the food industry towards utilizing vegetable proteins as substitutes or partial substitutes for animal-based proteins in a number of food formulations.

One of important functionalities of proteins as related to their applications in food formulations is their ability, as a kind of polymer emulsifiers, to facilitate the formation, improve the stability and provide some specific physicochemical properties to oil-in-water emulsions (McClements, 2004). It has been well recognized that vicilins exhibit better emulsifying properties than legumins, e.g. in the globulins from pea (Dagorn-Scaviner, Gueguen, & Lefebvre, 1987; Koyoro & Powers, 1987), soybean (Fukuda et al., 2005; Kimura et al., 2008) and fava bean (Kimura et al., 2008). The better emulsifying properties of vicilins have been attributed to their higher solubility (Koyoro & Powers, 1987), and surface hydrophobicity (Boye et al., 2010). On the other hand, some previous articles reported that some purified vicilins exhibit much better emulsifying properties (e.g. higher emulsifying activity index) than their protein isolates, e.g. from cowpea and pea (Rangel, Domont, Pedrosa, & Ferreira, 2003), and kidney bean (Yin, Tang, Wen, & Yang, 2010). These findings suggest that the vicilins or vicilin-rich proteins from *Phaseolus* legumes exhibit an excellent potential as a kind of emulsifiers to be applied in the food formulations.

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Due to differences in amino acid and/or polypeptide composition, surface properties and conformational characteristics (flexibility in tertiary and/or quaternary conformations), vicilins from different legumes considerably vary in their emulsifying properties (Kimura et al., 2008; Tang & Sun, 2011). Among vicilins (or 7S globulins) from pea, fava bean, cowpea, French bean and soybean, Kimura et al. (2008) reported that among the test vicilins from pea, Fava bean, cowpea, French bean and soybean, the vicilin from French bean (or kidney bean) exhibited best emulsifying properties, e.g., lower emulsion droplet size and much higher emulsion stability (especially at low ionic strengths). The excellent emulsifying ability of kidney bean vicilin has also been confirmed in our recent work as compared to two other vicilins from red bean and mung bean (Tang & Sun, 2011). However, we observed that the emulsion stability of kidney bean vicilin was significantly lower than that of the other two vicilins (as evaluated by the emulsion stability index, ESI; Tang & Sun, 2011), which seems to be contrasting from the Kimura et al.' findings (Kimura et al., 2008). The inconsistency might be associated with the differences in evaluation method for emulsifying properties, applied protein concentration (c; and oil fraction), as well as emulsification technique between these two previous works. More interestingly, we found that under the conditions applied in the work, e.g., at pH 7.0–9.0, the emulsifying ability (emulsifying activity index, or EAI) of these three test vicilins is closely related to the flexibility of their quaternary conformation, while the stability of the formed emulsions (emulsion stability index, or EAI) is largely dependent on the flexibility of their tertiary conformation (Tang & Sun, 2011). However, one point should be to clarify that these works were performed at a specific c value. Furthermore, the characteristics of the formed emulsions, including surface charge, emulsion size, flocculated state of oil droplets, and emulsion stability, are little characterized.

Surprisingly, the importance of c in the continuous phase for emulsifying properties of vicilins has been seldom addressed. The kinetic stability of protein-stabilized emulsions, e.g. creaming, flocculation and/or coalescence, are not only related to density difference between the dispersed and the continuous phases, but also dependent on inter-droplet interactions, and structure and viscoelastic properties of the interfacial protein films (Damodaran, 2005). Makri and Doxastakis (2006) observed that flocculation stability of emulsions stabilized by protein isolates from two *Phaseolus* legumes was accelerated by increasing c from 1.0 to 3.0%, upon storage of 1–70 days. Lawal, Adebowale, Ogunsanwo, Sosanwo, and Bankole (2005) observed that emulsifying activity and emulsion stability of a globulin from African locust bean were dependent on the c in the range 1–10%. Zhang, Jiang, Mu, and Wang (2009) also observed that EAI (at oil fractions of 4–12%, w/v) of chickpea protein isolate was highly dependent on the c ranging from 0.1 to 1.2%. Thus, it is very necessary to systematically investigate the c dependence of emulsifying properties for vicilins or vicilin-rich protein isolates.

Most of the physicochemical and conformational properties of vicilins are largely determined by their amino acid composition (especially relative acidic/basic charged amino acid ratio), an intrinsic physicochemical parameter (Tang & Sun, 2011). Higher acidic/basic charged amino acid ratio of vicilins seems to be associated with less flexibility of their tertiary conformation, or thermal stability, as well as higher flexibility of their quaternary conformation. In contrast, the secondary conformation of vicilins seems to be independent of the vicilin species (Tang & Sun, 2011). Among the three test vicilins, kidney bean vicilin exhibits highest acidic/basic charged amino acid ratio (2.19), and the vicilin from mung bean least (1.73). Based on these knowledge about the physicochemical and conformational characteristics of vicilins from kidney, red and mung beans, the present work was to systematically characterize the emulsifying and interfacial properties of these vicilins, especially the c dependence (in the c range 0.25–2.5%) of these properties, with the aim to understand the relationship between emulsifying properties and conformational characteristics of these vicilins. The emulsifying ability was evaluated using droplet size analysis, while the emulsion stability (against flocculation and/or coalescence, and creaming) upon storage of 24 h (or 2 weeks) was assessed by the droplet size (in water or 1% SDS) analysis and visual observation. The microstructure of droplets in the emulsions or cream layers was evaluated using confocal laser scattering microscope (CLSM). Furthermore, some physicochemical or interfacial characteristics, e.g., ζ-potential and interfacial protein concentration, and adsorption dynamics at the interface, were also characterized.

2. Materials and methods

2.1. Preparation of vicilins

Red kidney bean (*Phaseolus vulgaris* L.), red bean (*Phaseolus angularis*) and mung bean (*Phaseolus aureus*) were purchased from a local supermarket (Guangzhou, China). The seeds were soaked in de-ionized water for 12 h at 4 °C and de-hulled manually. The de-hulled seeds were freeze-dried, ground and defatted by Soxlet extraction with hexane to produce defatted flours. Vicilins from these beans were prepared according to a process described by Hall, McLeester, and Bliss (1977) with slight modifications. The defatted flours (5.0%, w/v) were dispersed in 0.5 M NaCl solution containing 0.025 M HCl (pH 3.5). The resultant dispersions were gently stirred at 25 °C for 2 h. The slurries were centrifuged (9000 *g*, 20 min) at 4 °C in a CR22G centrifuge (Hitachi Co., Japan), and the resultant supernatants were diluted with 5-fold volumes of deionized water (0–4 °C). Then, the precipitates were collected by centrifugation at 12,000 *g* for 20 min at 4 °C. The pellets were dissolved in 0.5 M NaCl solution, and re-precipitated twice as the above. The last obtained precipitates were finally dissolved in 0.5 M NaCl solutions and dialyzed against deionized water at 4 °C for 48 h, and then lyophilized to produce the vicilin samples (denoted as KV, RV and MV for the vicilins from kidney, red and mung beans, respectively). The protein content of these vicilins was 82.1, 82.1 and 85.1% for KV, RV and MV, respectively, as determined by Dumas method with a nitrogen conversion factor of 6.0.

2.2. Emulsion preparation

Various vicilin solutions at with c values of 0.25–2.5% (w/v) were prepared by dissolving the individual samples in 5 mM phosphate buffer (pH 7.0), and stirred using a magnetic stirrer for 2 h at room temperature, and then stored overnight at 4 °C to allow complete hydration. Sodium azide (0.02%, w/v) was used as an antimicrobial agent. Each protein solution was mixed with soy oil at oil fraction ($ø$) = 0.1, and pre-homogenized using the high-speed dispersing and emulsifying unit (model IKA-ULTRA-TURRAX® T25 basic, IKA® Works, Inc., Wilmington, NC) at 15,000 rpm for 1 min. Then, the pre-homogenized dispersions were further homogenized through a Microfluidizer (M110EH model, Microfluidics International Corporation, Newton, MA) for one pass at a pressure level of 40 MPa. The obtained fresh emulsions were subject to the following analyses, or stored at room temperature for various periods of time (e.g., 24 h) prior to the further analyses.

2.3. Droplet size determination

The droplet size distribution and volume-average droplet size ($d_{4,3}$) of various freshly prepared or stored emulsions were