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SHORT COMMUNICATION

Physicochemical characteristics of soy protein isolate and fenugreek gum dispersed systems

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Abstract Protein–polysaccharide interactions play an important role in the structure and stability of many processed foods. In this study the effect of fenugreek gum (**FG**) on solubility and emulsifying properties of soy protein isolate (**SPI**) was evaluated. Generally, the solubility of SPI ions was increased by adding FG. The emulsifying activity of SPI with fenugreek gum (**SPI-FG**) was 4 times higher than that of SPI or FG alone and similar to that of bovine serum albumin (**BSA**). The emulsifying stability of SPI-FG dispersions was respectively 3 and 2 times higher than that of SPI and BSA. In addition, the solubility and emulsifying properties of SPI-FG dispersions were stable over wide range of pH (from 3 to 9), ion strength (from 0.1 to 1.0 M NaCl) and high temperature (85 °C/h).

Keywords Fenugreek gum · Soy protein isolate · Emulsifying activity · Emulsion stability

Introduction

Food hydrocolloids play an important role in controlling and adjusting the rheological properties (e.g., viscosity and elasticity) of liquid and solid food products. Since the functions of the hydrocolloids relate directly to the textural or organoleptic properties of the products as well as indirectly to their flavor release, they are expected to be ingredients to create novel foods.

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The use of soy proteins (**SP**) as functional ingredients in food manufacturing is increasing because of their role in human nutrition and health. The major globulins of SP are conglycinin (7S) and glycinin (11S). Native SP, because of its quaternary and compact tertiary structure has limited foaming (Utsumi et al. 1997) and emulsifying (Liu et al. 1999) properties. However, structural modifications by chemical methods such as deamidation, succinilation and denaturation, allowing greater conformational flexibility of protein, may improve its surface behavior and functionality (Martinez et al. 2007, Ahmad et al. 2010).

Polysaccharides (**PS**) are used in admixture to proteins mainly to enhance stability of dispersed systems. Most high-molecular weight PS, being hydrophilic, do not have much of tendency to adsorb at the air–water interface, but they can strongly enhance the stability of protein foams by acting as thickening or gelling agents (Dickinson 2003). Above the protein isoelectric point thermodynamic incompatibility between the protein and PS generally occurs because of the repulsive electrostatic interactions and different affinities towards the solvent (Tolstoguzov 1997). There are some recent works in conditions of limited thermodynamic compatibility between the protein and PS (i.e. above the protein isoelectric point in the diluted concentration region) that support the evidence of interactions between proteins and PS at fluid interfaces (Baeza et al. 2004, 2005a, b; Martinez et al. 2007).

Gelling properties and other functional properties of food proteins are modified in the presence of hydrocolloid gums (Huaa et al. 2003). The gelation behavior of protein-polysaccharide mixtures generally fall into three patterns: formation of covalent bonds between two polymers; polyanion-polycation electrostatic interactions; and formation of composite gel due to mutual exclusion of each component (Morris 1990). Most protein-polysaccharide

mixtures are thermodynamically incompatible systems at neutral pH and the mixture can be separated into two liquid phases according to phase diagrams. After gelation of the incompatible polymer mixture, the resulted gels could be regarded as filled or composite gels. The rheological properties of composite gels could be predicted from the behavior of individual constituents by applying the blending laws (Clark 1987).

Protein and polysaccharide are two main types of food polymers often used in oil in water emulsions (Dickinson and Euston 1991). Generally, proteins stabilize emulsions by forming an adsorbed layer at the oil-water interface from a protective steric harrier around oil droplets (Dickinson 1994a). The addition of polysaccharides affects emulsion stability considerably through rheological modification of the aqueous medium and interaction with protein-coated droplets (Dickinson 1994b). The application of protein-polysaccharide dispersions on emulsifiers could create new foods with low oil content and high nutritional value (Dalev and Simeonova 1995).

Fenugreek (*Trigonella foenum-graecum*) is an annual herb belonging to the legume family; it is widely grown in Middle Eastern countries. The seeds are used as a spice and the leaves are consumed as a green vegetable. Over the last years, research interest in extractable seed components, such as storage carbohydrates and saponins have been extended (Flammang et al. 2004). The seeds have also been reported to improve diabetic conditions (Basch et al. 2003), exhibit antioxidant properties (Kaviarasan et al. 2004), and control plasma cholesterol level (Madar and Shomer 1990, Vidyavati et al. 2010).

Fenugreek gum (**FG**) is derived from the endosperm of the seeds. Fenugreek gum, like guar gum and locust bean gum, is composed of galactose and mannose, and gives a high viscosity in aqueous solutions. The galactomannans are neutral water-soluble polysaccharides, widely used as thickening, water holding, stabilizing, and emulsifying agents in food products (Brummer et al. 2003). Structurally they are composed of a linear backbone of β-D (1→4) D-mannose units with α-D (1→6) D-galactose units attached as side chains (Evans et al. 1992). Fenugreek gum has a uniform distribution of galactose to mannan backbone in the ratio of 1:1 or in a few cases,1:2 (Garti 1999). This highly branched structure may explain the lower viscosity of fenugreek gum when compared to other galactomannans (such as guar gum and locust bean gum) at the same molecular weight and concentration (Cui 2001). Fenugreek gum (**FG**) exhibited the highest stabilizing properties among 11 commercial gums and five laboratory prepared gums in an oil/water emulsion model system. Salini and Sudesh (2004) reported that the addition of 10% of fenugreek flour to wheat flour increased protein content, fibre, total calcium and total iron; this indicates that fenugreek can be incorporated to prepare acceptable biscuits, and may also be mixed with cereals as a supplement for some limiting amino acids (El-Nasri and El-Tinay 2007).

Given that fenugreek is a minimally researched food gum, a complete understanding of the physical properties and structure of FG will shed light on the structural origin of its properties and aid in the eventual utilization of FG in the food industry. The ability of FG as stabilizer and thickener has not yet been disclosed and practically has not been studied. The aim of this work was to evaluate the solubility and emulsifying properties of dispersions prepared from SPI with FG (**SPI-FG**) and to study the effects of different conditions including pH, salt and heat treatment on the functional properties of SPI-FG dispersion systems.

Material and methods

Materials

Seeds of fenugreek (*Trigonella foenum-graecum*) were purchased from the local market (Zagazig, Egypt). Bovine serum albumin (**BSA**) and other reagents were purchased from Merck (Darmstadt, Germany).

Preparation of fenugreek gum (FG)

Fenugreek gum was prepared from dry seeds of fenugreek according to the method of Garti et al. (1997). The seeds (125 g) were ground to fine powder (300 mesh) and extracted by the Soxhlet procedure in the presence of 2× 100 mL *n*-hexane for 5 h. The *n*-hexane extract that contained lipids (1.5 g) was discarded. The solid fraction was further extracted with ethanol (200 mL) followed by methanol (150 mL). The extract was vacuum evaporated and lyophilized to yield 7–8 g of a mixture of saponins. The remaining solids were further treated and dissolved in water (800–1,000 mL) to form a viscous aqueous solution of crude fenugreek gums. First centrifugation (5,000 rpm) precipitated the non-soluble cellulose, hemicelluloses, lignin and part of the proteins. Second centrifugation (100.000 rpm) precipitated most of the residual proteins and other non-soluble fractions. The extraction process was repeated four times. The water soluble fractions were combined and the hydrocolloid fractions were precipitated by adding ethanol (1:1, w/w).

Preparation of SPI

The SPI was prepared from defatted soybean flour according to Wolf and Cowan (1975). Soybeans were cracked in a

blender, dehulled in a vertical aspiration unit and milled to provide flour. The soybean flour was defatted twice in *n*-hexane for 10 min (1:3, flour:hexane) and dried overnight under a hood to remove residual *n*-hexane. Defatted soybean flour was suspended in water (1:10, flour:water) followed by alkaline extraction at pH 9.0 for 30 min after centrifugation at 10.000 rpm for 10 min. The supernatant pH was adjusted to 4.5 (isoelectric point of soy protein) with 1 N HCl to precipitate proteins, followed by centrifugation at 10.000 rpm for 10 min. The protein precipitate was washed with water and pH was adjusted to 7 then freeze-dried and stored at 5 °C until used.

Preparation of SPI-FG dispersions

Six portions of SPI (1 g) and 0.3, 0.5, 1, 2, 3 or 4 g of FG were mixed in a dry state manually to prepare the respective SPI-FG blends in the weight rations of SPI/FG=3:1, 2:1, 1:1, 1:2, 1:3 and 1:4. Six of SPI-FG dispersions were prepared by transferring the blends into 0.1 M sodium phosphate buffer (pH 7.4) while stirring with a magnetic stirrer for 60 min at ambient temperature to provide dispersion with 0.1% soy protein. These dispersions were used for determination of viscosity and emulsifying properties. Based on preliminary data, SPI-FG dispersions in the weight ratio of 1:2 which gave the optimum emulsifying properties were chosen to evaluate effects of pH, heat and salt treatments.

Determination of viscosity

The viscosity of SPI-FG dispersions in 0.1 M sodium phosphate buffer (pH 7.4) was measured by Hobbler viscometer. All measurements were carried out at ambient temperature 24±1 using spindle #3 at a speed of 20 rpm.

Nitrogen solubility determination

Nitrogen solubility (**NS**) of SPI-FG dispersions was determined by the dye-binding method of (Bradford 1979). SPI-FG dispersions were prepared by slowly transferring the blends into distilled deionized water to give dispersions containing 0.1% soy protein. The pH of these dispersions containing 0.1% soy protein was adjusted to 7 and stirred with a magnetic stirrer for 60 min at ambient temperature then centrifuged at 5,000 rpm for 15 min. Coomassie blue reagent (5 mL) was added to 100 μL of SPI-FG solution. The tubes were vortexed and let stand at room temperature for 30 min. Nitrogen content of the supernatant was determined by absorbance at 595 nm using the related standard curve. The standard curve was prepared by using SPI solution at pH 12 (at this pH, SPI is completely dissolved)

The percent NS was calculated as follows:

$$\mathrm{NS}\% = \frac{\text{Nitrogen in supernatant}}{\text{Total nitrogen in sample}} \times 100$$

Determination of emulsifying properties

The BSA (0.1%, w/v), SPI (0.1%, w/v), FG (0.2%) and SPI-FG dispersions were used to measure emulsifying properties according to Pearce and Kinsella (1978). Corn oil (2 mL) and 6 mL of 0.1% protein solution were homogenized in a mechanical homogenizer at a setting of 6 for 1 min. Emulsion (50 μL) was taken from the bottom of the container at 0 and 10 min and mixed with 5 mL of 0.1% sodium dodecyl sulfate SDS. Absorbance of the emulsions was measured at 500 nm. Absorbance measured immediately after emulsion formation (0 min) was expressed as emulsifying activity (EA). The emulsifying stability (ES) was determined as follows.

$$\mathbf{ES} = \mathbf{T}/\mathbf{T_0}$$

(Where T_0 and T are turbidities at 0 and 10 min, respectively)

Just after preparation, measured amounts of emulsions were poured into glass tubes and stored in a refrigerator at 4 °C. The height of the clear liquid at the bottom was measured after 24 h and recorded to indicate creaming stability.

Salt, pH and heat treatment

The SPI-FG dispersions (0.1% SPI and 0.2% FG) were prepared by solubilizing 0.06 g SPI-FG bland (SPI/FG=1/2) in 20 mL of NaCl solutions containing 0.1 M, 0.5 M and 1.0 M NaCl (pH 7.4). These solutions were used to determine solubility and emulsifying properties.

For pH treatments, the SPI-FG dispersions (0.1% SPI and 0.2% FG) were prepared by solubilizing 0.242 g SPI-FG blend (SPI/FG=1/2) in 80 mL of distilled water and divided equally into 4 batches. The pH values of SPI-FG dispersions were adjusted to 3.0, 4.5, 7.0, 9.0 with 0.1 M NaOH and HCl then solubility and emulsifying properties were determined.

For heat treatments, the SPI-FG dispersions (SPI/FG=1/2) in 0.1 M sodium phosphate buffer (pH 7.4) were heated at 85 °C for 60 min, cooled to ambient temperature and evaluated for solubility and emulsifying properties.

Statistical analysis

Statistical analyses were performed by using General Linear Models (GLM) procedure of analysis of variance

Table 1 Effect of fenugreek Gum (FG) levels on nitrogen solubility (NS), emulsifying activity (EA), and emulsifying stability (ES) of soy protein isolate (SPI)

Sample	Nitrogen solubility	Emulsifying activity	Emulsifying stability
SPI	48.51	0.11	25.09
SPI/FG (2:1)	50.1	0.65	11.5
SPI/FG (1:1)	58.5	0.897	81.6
SPI/FG (1:2)	83.6	1.092	98.5
SPI/FG (1:3)	83.2	1.061	94.7
SPI/FG (1:4)	81.7	0.876	93.8
FG	0	0.152	80
Bovine serum albumin	0	1.14	37.4

Emulsion were prepared in phosphate buffer pH 7.4 at room temperature

(SAS Institute 1994). Least significant difference (LSD) values were computed with significance defined at ($p>$ 0.05). Experiments were performed in triplicate.

Results and discussion

The emulsifying action of soy protein isolate (**SPI**) does not always provide emulsions of effective activity and stability. In many cases, a certain degree of modification is needed so that soy protein could meet different requirements of processed foods. Protein–polysaccharide interaction could be a simple and safe way of modifying the functional properties of soy protein. Soy protein–gum mixed gels have found practical applications in manufacturing of imitation cheeses. Studies also demonstrated that the addition of polysaccharide gums could increase the water binding capacity of soy proteins, improve textural quality of tofu (Karim et al. 1999) and help retaining isoflavones in soy protein concentrate (Huaa et al. 2003).

Effect of FG on nitrogen solubility (NS) of SPI

The effects of FG on NS of SPI were presented and compared in Table 1. The NS of SPI was increased by addition of FG ($\rho<0.05$). No further increase in NS of SPI-FG dispersion was observed after increasing SPI/FG ratio beyond 1:2. The NS of SPI-FG dispersions was stable over a wide range of ionic strength (0.1 to 1.0 M NaCl), pH (3.0 to 9.0), and heat treatment (85 °C, 1 h) (Table 2). Protein-polysaccharide complex formation has been applied to increase protein solubility (Payens 1972). This type of interaction also inhibits precipitation of some water-soluble proteins following heat denaturation (Hidalgo and Hansen 1969) and at pH values in the usual isoelectric range. Generally, initial solubility of proteins must be relatively high for improving functionality in emulsions and foams. The initial solubility facilitates protein diffusion to air/water and oil/water interfaces, thus improving surface activity.

Effect of FG on emulsifying properties of SPI

The emulsifying activity (EA) and emulsifying stability (ES) of SPI-FG dispersions with various SPI/FG ratios (3:1, 2:1, 1:1, 1:2, 1:3 and 1:4), and BSA preparations were compared in Table1. BSA was used as a standard to compare emulsification properties of proteins because of its good emulsifying properties. The EA and ES of SPI-based emulsions were improved by the increase addition of FG ($p<$ 0.05). The optimum EA and ES of SPI-FG dispersions were observed in the weight ratio of 1:2. No further improvements in EA and ES were observed by further increasing SPI-FG ratio beyond 1:2. The optimum EA of SPI-FG dispersions was 4 times higher than that of SPI or FG alone, and similar to that of BSA ($p<0.05$). The optimum ES of SPI-FG dispersions was 3 and 2 times higher than that of SPI and BSA ($p<0.05$), respectively. NS of SPI-FG dispersions at different weight ratio were correlated with EA ($r=0.96$, $p<$ 0.001) (Fig. 1). Improved ES of SPI-FG dispersions were associated with viscosity increase as presented in Fig. 2. A sharp increase in ES of SPI-FG was related to viscosity increase from 50 to 110 cps.

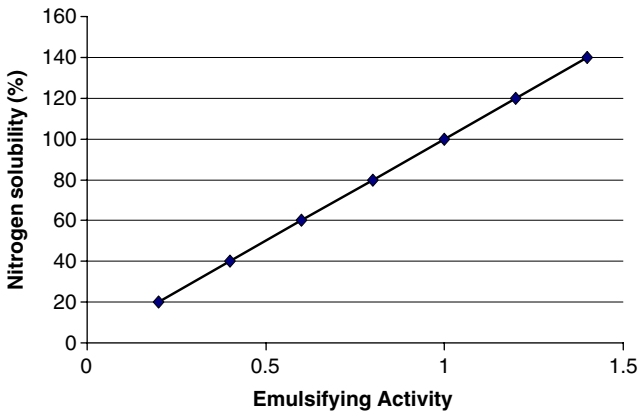

Fig. 1 Correlation between the emulsifying activity and nitrogen solubility of dispersions containing soy protein isolate and fenugreek gum

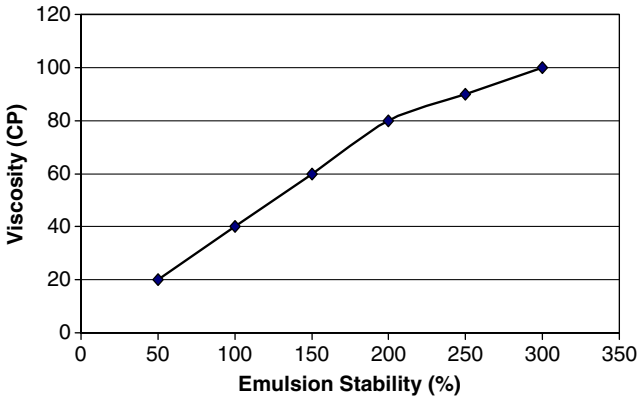

Fig. 2 Correlation between the emulsion stability (%) and viscosity of dispersions containing soy protein isolate and fenugreek gum

Nitrogen solubility appears to be the most important factor affecting the EA of proteins (Halling 1981). Generally, protein solubility has positively correlated with emulsifying activity. Undissolved proteins make little or no contribution to emulsification, because proteins must dissolve and migrate to the interface before their surface properties are active. Results indicated that increased NS of SPI due to addition of FG contributed to enhance EA of SPI based emulsions. In emulsion systems containing both protein and polysaccharide, protein typically form an adsorbed primary layer at the oil water interface. Hydrophilic polysaccharides possibly form a thick secondary layer enhancing steric stabilizing properties on the outside of protein-coated droplets (Dickinson 1994b).

The protein adsorption is intensified and the protein content required for a multilayer adsorption is reduced by addition of a polysaccharide. This is due to increased charge interaction on the surface of adsorption layers due to high charge density on the polysaccharide molecule. Also there is better contact between protein and polysaccharide molecules due to an altered conformation of protein at low adsorption. In soy oil water emulsions, FG contributed to the ES by its adsorption at the oil/water interface, thus lowering surface tension. It also formed liquid crystalline lamellae in the continuous water phase, trapping the emulsion droplets in the microgel matrix (Samant et al. 1993).

Effects of salt on emulsifying properties of SPI-FG

SPI-FG emulsions were equally stable over a wide range of NaCl concentrations (0.1 to 1 M, pH 7.4) ($p<0.05$) as presented in Table 2. For SPI-FG, the excellent compatibility between these two biopolymers over a wide range of NaCl concentrations indicated that the affinity between the biopolymers was non-electrostatic. Similar results between serum albumin and pectin were reported by Samant et al. (1993). The formation of micellar structure would better explain SPI-FG compatibility, which is similar to that reported between serum albumin and pectin. The stable NS of SPI-FG dispersions over a wide range of ionic strengths (0.1 to 1.0 M NaCl) and salt compatibility of FG were due to its rigid helical conformation, hydrogen bonded dispersions, and anionic charge onside chains. These may contribute to the stable emulsifying proper ties of SPI-FG dispersions over the wide range of ionic strengths.

Effects of pH on emulsifying properties of SPI-FG

Soy protein isolate (SPI) has good gelation, emulsifying, foaming and water absorption properties (Utsumi et al. 1997), however, it exhibits poor solubility within the acidic pH regions. The EA and ES of SPI-FG was stable over the range of pH 3.0 to pH 9.0 (Table 2). At pH 4.5, the isoelectric point (**IP**), SPI was almost insoluble, which would not favor emulsification. Proteins in this state do not contribute to the stabilizing repulsive. Soy proteins have

Table 2 Effect of pH (3.0-9.0), heat 85 °C, salt (0.1–1.0 M NaCl) treatments on the nitrogen solubility (NS), emulsifying activity (EA) and emulsion stability (ES) of dispersions containing soy protein isolate and fenugreek gum

Treatments		Nitrogen solubility	Emulsifying activity	Emulsifying stability
pH[a]	3.0	80.2	1.05	97.5
	4.5	82.0	1.07	96.8
	7.0	80.5	1.02	97.1
	9.0	79.7	1.10	96.6
Heat (85 °C)[b]	60 min	81.3	1.07	97.2
NaCl (M)[c]	0.1	83.7	1.11	97.5
	0.5	81.1	1.03	96.7
	1.0	80.9	1.07	97.3

[a] Emulsions were prepared in phosphate buffer (pH 7.4) at room temperature

[b] Emulsions were prepared in 0.1 M phosphate buffer (pH 7.4)

[c] Emulsions were prepared at room temperature (pH 7.4)

better emulsifying properties at pH values away from the IP, wherein higher electrostic and hydration repulsion forces occur. The excellent stability of SPI-FG emulsions over the range of pH 3.0 to pH 9.0 indicates that FG probably formed a protective layer around SPI coated droplets. This resulted in enhancing the stabilizing properties of SPI based emulsion and prevented SPI perspiration at IP. The stable NS of SPI-FG dispersions and stability of FG to acid and alkali due to its backbone protected by overlapping side chains may contribute to the stable emulsifying properties of SPI-FG dispersions over the wide pH range. Carp et al. (1999) have shown that under conditions of neutral pH where a limited thermodynamic compatibility between soy protein and xanthan exists, xanthan promoted soy protein subunits aggregation at the air–water interface in foams based on native soy protein, but promoted the basic B-11S polypeptide to be predominating at the interface of denaturated SP foams. Those specific effects should further influence the interfacial and foaming properties of the mixed systems. Because the emulsifying properties of most commercial emulsifiers decrease greatly at low pH, the stable emulsifying properties of SPI-FG at acidic pH would be useful in industrial applications, such as beverages and dairy products.

Effect of heat treatment on emulsifying properties of SPI- FG

The EA and ES of SPI-FG were stable after heat treatment at 85 °C for 1 h ($p>0.05$) as shown in Table 2. The ES of protein-stabilized emulsions usually decrease during heating because of the decrease in viscosity and the rigidity of the protein film adsorbed at the interface. In contrast, protein polysaccharide interactions inhibit precipitation of some water soluble proteins following heat denaturation (Hidalgo and Hansen 1969). The stability under heat treatment (85 °C for 1 h) further supports the hypothesis that FG forms a protective layer around the outside of SPI coated droplets inhibiting or preventing heat denaturation and aggregation of SPI and stabilizing the emulsion. The pseudoplastic nature of FG solutions stabilizes emulsions at elevated temperatures. This heat resistance property would be useful in heat pasteurization for food applications such as dairy products.

Conclusion

Many researchers have attempted to convert food proteins into useful proteins with better functional properties through physical, chemical and/or enzymatic treatments. However, most of these methods utilize toxic chemical products and are not permitted for potential industrial applications. Significant improvements in the solubility and emulsifying activity of SPI were achieved by mixing with FG. In addition, the solubility and emulsifying properties of SPI-FG dispersions were stable over wide range of pH, ion strength and heat. The results demonstrate the possibility of exploiting combinations of FG with SPI to improve functional properties and modify the solubility of SPI.

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