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# Physicochemical properties of dry-heated soy protein isolate–dextran mixtures

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**Abstract**

Soy protein isolate–dextran mixtures were incubated for up to three weeks and an improvement of their ability to stabilize emulsions against creaming, giving emulsions with lower droplet size, at pH values near the protein isoelectric point, was observed. Analysis of the adsorbed protein fraction indicated that the protein–polysaccharide hybrid can adsorb, together with the other mixture constituents, at the interface, during emulsion formation and in this way may contribute to droplet stabilization by enhancing repulsive steric forces by interpenetrating neighbouring droplets and polysaccharide chains.
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## 1. Introduction

Maillard-type reactions of several proteins with polysaccharides, leading to covalent bond formation under benign reaction conditions, i.e., by gently heating of a dry biopolymer mixture over a period of few weeks, has been exploited by a number of workers in order to prepare hybrids with superior emulsifying properties compared to non-treated mixtures (Dickinson, 1993; Kato, Sasaki, Furuta, & Kobayashi, 1990; Kato, Shimokawa, & Kobayashi, 1991; Shepherd, Robertson, & Ofman, 2000). The conjugates are thought to owe their improved emulsifying and emulsion stabilizing properties to a combination of the adsorption ability of their protein moiety (which, due to molecular hydrophobicity and flexibility, is able to rapidly adsorb and rearrange at the oil–water interface resulting in the formation of a coherent protective molecular film) and the high hydrophilicity of the polysaccharide part of the conjugate leading to the formation of a strongly solvated layer near the oil–water interface, which confers steric stabilization to emulsion oil droplets (Dickinson & Galazka, 1991).

Soy proteins are important ingredients in many food formulations where they extend or replace the functionality of animal proteins in these systems. As Kobayashi, Kato, and Matsudomi (1990) reported, when soy protein isolate (SPI)–dextran mixtures are dry-heated at 60 °C for up to four weeks, a protein–polysaccharide may form, exhibiting increased solubility, and its emulsifying properties, as assessed by turbidity measurements, are superior compared to those of the SPI–dextran mixture. Turbidity measurements, however, are of limited value when emulsion properties, such as oil droplet size, droplet coalescence and creaming behaviour are under consideration, since differences in turbidity between emulsions may not only reflect differences in droplet size but also in the state of the oil droplet and/or polymer aggregation. As Dickinson and Galazka (1991) observed, for emulsions stabilized by heat-treated β-lactoglobulin–dextran mixtures, the mean droplet size, $d_{3,2}$, was in fact rather larger than that of emulsions prepared with the pure native protein and marginally lower compared to those of emulsions stabilized by the protein–polysaccharide mixture. What is

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also important to know is the way the various components of the heat-treated SPI–dextran mixture behave during emulsification and affect the physicochemical properties of the system, in view of the fact that (Diftis & Kiosseoglou, 2003), when soy-protein–carboxymethylcellulose mixtures are dry-heated, the soy protein constituents exhibit different reactivities with the polysaccharide, resulting in a mixture of a protein–polysaccharide hybrid and pure protein components. The aim of this work, therefore, was to investigate the emulsifying and adsorption behaviour of heated SPI–dextran mixtures in order to extend our knowledge about the way the biopolymer components of the composite behave during oil emulsification.

## 2. Materials and methods

### *2.1. Materials*

Medium molecular weight dextran (188KDa) was purchased from Sigma (St. Louis, MO). Soybeans were provided by the Cotton Research Institute (Thessaloniki), while commercially refined corn oil was obtained from a local supermarket and was used without further purification.

### *2.2. Preparation of the SPI–dextran conjugates*

Soybean protein isolate was first obtained from soybeans by dispersing (comminuted and extracted with petroleum ether) soybeans in distilled water at pH 9 and stirring the mixture for 2 h. The protein was recovered by precipitation at pH 4.5, washed with distilled water, the pH was adjusted to 7 and the precipitate was finally freeze-dried. Its protein content determined by the Kjeldahl method (AOAC, 1994) was 91% w/w, ($N \times 6.25$).

Protein–polysaccharide dry mixtures of 1/1 and 1/3 (w/w) were prepared by mixing solutions of the two biopolymers, followed by freeze-drying and comminution. The resulting fine powders were then incubated at 60 °C in a desiccator in the presence of saturated KBr solution, as described by Kato, Minaki, and Kobayashi (1993). Samples, drawn after incubation for 1 and 3 weeks, were freeze-dried and analyzed by SDS–PAGE. Their free amino and sulfhydryl group contents were also determined.

### *2.3. SDS–PAGE analysis*

Soybean protein and SPI–dextran mixtures were analyzed according to Laemli (1970) using 10% and 1% (v/w) acrylamide separating and stacking gel, respectively. Following staining of the gel sheets with Coomassie brilliant blue G-250, the gels were photographed with a Kodak digital camera and the photographs were analyzed using the Gel Pro Analyzer (Version 3, Media Cybermetics 1993–1997) scanning densitometer software to determine the amount of each protein band.

### *2.4. Free amino and sulfhydryl group content and protein solubility*

The free amino and the sulfhydryl group contents of the samples were determined by the trinitrobenzenesulfonate (Haynes, Osuga, & Feeney, 1967) and the Ellman's reagent method (Beveridge, Toma, & Nakai, 1974), respectively.

Protein solubility was determined by dispersing the samples in distilled water to obtain final solutions of 0.5% (w/w) in protein. Following adjustment of pH 9 and agitation for 1 h with the aid of a mechanical stirrer, the pH was readjusted to 6.5 or 3.8 and the clear protein solutions obtained by centrifugation at 3500*g* for 30 min were analyzed for protein by the Lowry method (Lowry, Rosenbrough, Farr, & Randall, 1951).

### *2.5. Emulsifying properties*

The continuous phase (0.5% w/v in protein) of emulsions was first prepared by vigorous and continuous agitation of protein–polysaccharide samples in distilled water of pH 8.5 with the aid of a mechanical stirrer (RW20.n IKA, Labortechnik, Germany) equipped with a Dayton D15 (10 mm) propeller and sodium azide (0.01%) was added to retard microbial growth. Then the pH was decreased to 6.5 or 3.8. Oil-in-water (o/w) emulsions were prepared by adding 5 ml of corn oil in 45 ml of protein–polysaccharide solution under continuous agitation and the resulting crude emulsions were homogenized for 1 min using an Ultra-Turrax T25 (IKA Labortechnik, Germany) homogenizer (rotor stator principle), equipped with a dispersing tool (S25KG-25F), operated at 25,000 rpm. The oil droplet size distributions were evaluated with dynamic light scattering using a Malvern Mastersizer 2000 unit (Malvern Instruments, UK). The instrument settings were: corn oil refractive index, 1.4673; absorption, 0.002; water refractive index, 1.33. The stability of emulsions against coalescence was evaluated by determining the average oil droplet size following storage of the samples at room temperature for 50 days. The stability against creaming was determined visually from the serum separated in samples stored in glass containers for up to 10 days.

### *2.6. Adsorption behaviour of protein–polysaccharide samples*

The surface protein load, $\Gamma_s$, expressed as the amount of protein adsorbed per unit of interface (mg/m$^2$), was determined by centrifugation at 2000*g* for 30 min of

the emulsion samples, washing the isolated cream with a phosphate buffer of a pH similar to that used in the preparation of the emulsion (6.5 or 3.8). The clean and unaffected cream, which contained the whole adsorbed protein was collected and transferred to a new plastic tube. Its oil droplets were then broken up, by applying repeated freeze–thaw cycles. The desorbed protein was then dissolved in 20 ml of Tris–SDS buffer (pH 8.8). The oil was separated easily with a second centrifugation (500*g*) for 10 min, and a sample of the resulting Tris-SDS solution was analyzed for protein by the Lowry method (Lowry et al., 1951) while its composition was assessed by SDS–PAGE.

The amount of protein adsorbed was calculated by applying the equation:

$$\Gamma_s\ (\mathrm{mg/m^2}) = \Gamma_T / S_T, \quad (1)$$

where $\Gamma_T$ is the total amount of protein adsorbed and $S_T$ is the total emulsion surface derived by the equation:

$$S_T = (\mathrm{ml\ oil}) \cdot S, \quad (2)$$

where $S$ is the average surface area ($\mathrm{m^2/mg}$ oil) calculated by using the equation (Walstra, 1983)

$$S = 6/d_{3,2}. \quad (3)$$

### 2.7. Statistical analysis

All experiments were conducted at least three times and the data were statistically analyzed using the one-way ANOVA programme. Significant differences between means were identified by the LSD procedure.

## 3. Results and discussion

As was expected, dry-heating of SPI–dextran mixture at 60 °C resulted in the formation of a Maillard-type protein–polysaccharide hybrid (Fig. 1), which appears as high molecular constituents at the beginning of the stacking and separating gels. Analysis of the SDS–PAGE electropherograms indicated that the greatest proportion of chemical changes took place within 1 week of heat treatment while the decrease in the amount of soy protein constituents, as a result of protein–dextran conjugate formation, was much slower thereafter (Table 1). It appears that all soy protein constituents took part in the reaction, although the basic subunits of the 11S fraction reacted to a lesser extent in the case of a 1/1 protein/polysaccharide ratio, possibly due to their much lower lysine content (Utsumi, Matsamura, & Mori, 1997). When, however, the ratio was 1/3 they also interacted to the same extent as the 7S fraction subunits since the available carbonyl sites were more abundant. In contrast, however, to SPI–NaCMC mixtures (Diftis & Kiosseoglou, 2003) where the basic

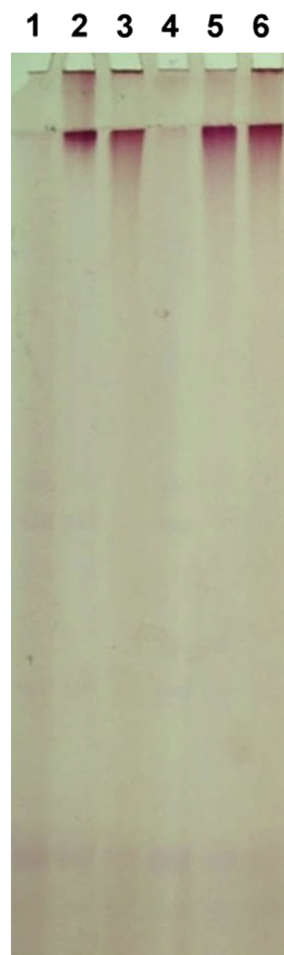


Fig. 1. SDS–PAGE patterns for SPI–dextran mixtures incubated at 60 °C for up to 3 weeks, stained for carbohydrate. Lanes 1–3: incubation for 0, 1, 3 weeks, respectively, protein/polysaccharide weight ratio 1/1. Lanes 4–6: incubation for 0, 1, 3 weeks, respectively, protein/polysaccharide weight ratio 1/3.

subunits did not react at all with the polysaccharide, in the case of dextran they reacted to a significant extent, possibly because of the much higher flexibility of

Table 1
Protein composition (%)[a] of heat-treated at 60 °C for up to 3 weeks, SPI–dextran mixtures

| Protein constituent | Protein/polysaccharide ratio | | | | | |
|---|---|---|---|---|---|---|
| | 1/1 | | | 1/3 | | |
| | Incubation time (weeks) | | | | | |
| | 0 | 1 | 3 | 0 | 1 | 3 |
| Maillard products | 0.0 | 46.0 | 57.7 | 0.0 | 56.5 | 69.7 |
| 7S α′ sub. | 9.7 | 4.9 | 3.1 | 8.9 | 4.3 | 3.2 |
| 7S α sub. | 10.9 | 5.6 | 3.2 | 11.7 | 4.6 | 3.1 |
| 7S β sub. | 13.5 | 7.5 | 4.9 | 12.7 | 5.6 | 3.8 |
| 11S Acidic sub. | 35.0 | 16.9 | 12.9 | 35.8 | 13.5 | 8.7 |
| 11S Basic sub. | 26.4 | 16.1 | 15.8 | 26.7 | 12.4 | 9.2 |
| Other protein constituents | 4.5 | 3.0 | 2.4 | 4.2 | 3.1 | 2.3 |

[a] C.V.%: 8.4.

Table 2
Free amino and sulfhydryl group content of heat-treated (at 60 °C for up to 3 weeks) SPI and SPI–dextran mixtures

| Sample | Amino group content (μmol $NH_2$/g) | | | Sulfhydryl group content (μmol SH/g) | | |
|---|---|---|---|---|---|---|
| | Incubation time (weeks) | | | | | |
| | 0 | 1 | 3 | 0 | 1 | 3 |
| SPI | $28.0^{a}$ | $20.0^{b}$ | $16.6^{c}$ | $5.0^{a}$ | $4.3^{a}$ | $1.9^{b}$ |
| SPI–dextran (1/1) | $29.5^{a}$ | $26.4^{a}$ | $19.1^{b}$ | $5.3^{a}$ | $4.2^{a}$ | $3.4^{a}$ |
| SPI–dextran (1/3) | $28.4^{a}$ | $20.9^{b}$ | $13.2^{c}$ | $4.4^{a}$ | $4.0^{a}$ | $3.9^{a}$ |

a–c Means within a column and a row with same superscripts are not significantly different ($p < 0.05$).

dextran molecules compared to CMC. Although SPI–dextran conjugate formation during heat-treatment is indisputable, as can be seen by the SDS–PAGE electopherograms stained for carbohydrate, the possibility of development of other polymerization products, due either to isopeptide or to disulfide bond formation, cannot be excluded. As shown in Table 2, when SPI is dry-heated for a period of up to 3 weeks, a marked reduction in amino and sulfhydryl group contents takes place. In the presence of dextran, the decrease of free sulfhydryl group content is not significantly affected, possibly due to the protection of the protein from oxidation by the conjugated polysaccharide, which prevents the protein molecules from unfolding (Kato, Nakamura, Takasaki, & Maki, 1996).

Emulsions of pH 3.8, prepared with SPI–dextran mixtures incubated for 1 week, exhibited oil droplet sizes shifted to lower values than those containing non-treated mixtures while no significant differences were observed in the case of emulsions of pH 6.5, irrespective of the protein–dextran ratio or time of incubation (Table 3). These results are opposite to those obtained in the case of SPI–CMC mixtures, where the beneficial effect of protein–polysaccharide incubation was observed at pH 6.5 and not at 3.8 (Diftis & Kiosseoglou, 2003).

Protein solubility in the presence of dextran remains quite high, even after incubation for a period of up to three weeks (Table 4) leading to improved emulsifying ability.

Another consequence of protein–dextran conjugate formation during dry-heating is an enhancement of emulsion stability against creaming (Fig. 2), irrespective of the pH of the system. This should be attributed to the formation of an emulsion droplet network which, due to the presence of adsorbed polysaccharide molecules in the interdroplet region, may exhibit a yield value high enough to resist the forces favouring upward movement and creaming (Parker, Gunning, Ng, & Robins, 1995). When the polysaccharide molecules have not been conjugated to soy protein, as is the case of the non-treated mixture, they will tend to favour the process of creaming by their depletion flocculation effect (Dickinson, 1993).

Evidence for the presence of Maillard-type SPI–dextran conjugate at the o/w interfacial membrane is provided in Fig. 3, where SDS–PAGE electrophoresis zones stained for carbohydrate are shown. In the case of non-heat-treated protein–polysaccharide mixtures, SPI–dextran hybrids do not form and, therefore, they cannot be traced at the interface. Thus, all the polysaccharide molecules are located in the continuous phase where they may accelerate creaming of oil droplets. In the case of heat-treated SPI–dextran samples, dextran molecules are placed at the interface since they accompany adsorbed protein molecules and, in this way, enhance the stability at the dispersion system.

With respect to stability against droplet coalescence, as assessed by determining the oil droplet size of emulsions following storage for 50 days, it was observed that all the emulsions were very stable, irrespective of the presence of protein–polysaccharide mixture or of the hybrid. Although, in the case of emulsions stabilized with the incubated protein–polysaccharide mixture, steric forces due to adsorbed dextran molecules at the oil–water interface, may prevent the droplets from

Table 3
Mean droplet size, $d_{32}$ (μm), of o/w emulsions prepared with heat-treated (at 60 °C) SPI or SPI–dextran mixtures, determined one day after preparation

| Sample | pH 6.5 | | | pH 3.8 | | |
|---|---|---|---|---|---|---|
| | Incubation time (weeks) | | | | | |
| | 0 | 1 | 3 | 0 | 1 | 3 |
| SPI | $3.9^{a}$ | $4.6^{a}$ | $6.6^{b}$ | $14.5^{a}$ | $16.2^{b}$ | $16.8^{b}$ |
| SPI–dextran (1/1) | $3.2^{a}$ | $2.8^{a}$ | $4.7^{b}$ | $6.4^{b}$ | $3.3^{a}$ | $4.5^{a}$ |
| SPI–dextran (1/3) | $3.0^{a}$ | $2.8^{a}$ | $3.1^{a}$ | $8.3^{b}$ | $3.8^{a}$ | $3.3^{a}$ |

a,b Means for each pH within a row with same superscripts are not significantly different ($p < 0.05$).

Table 4
Protein solubility (%) of SPI and SPI–dextran mixtures heat-treated at 60 °C for up to 3 weeks

| Sample | pH 6.5 | | | pH 3.8 | | |
|---|---|---|---|---|---|---|
| | Incubation time (weeks) | | | | | |
| | 0 | 1 | 3 | 0 | 1 | 3 |
| SPI | $80.8^{a}$ | $56.8^{c}$ | $29.8^{d}$ | $55.8^{a}$ | $40.6^{b}$ | $11.9^{c}$ |
| SPI–dextran (1/1) | $83.8^{a}$ | $66.3^{b}$ | $50.1^{c}$ | $50.8^{a}$ | $49.9^{a}$ | $33.7^{b}$ |
| SPI–dextran (1/3) | $83.7^{a}$ | $66.2^{b}$ | $61.6^{b}$ | $53.0^{a}$ | $51.7^{a}$ | $46.1^{a,b}$ |

a,b Means for each pH with same superscripts are not significantly different ($p < 0.05$).

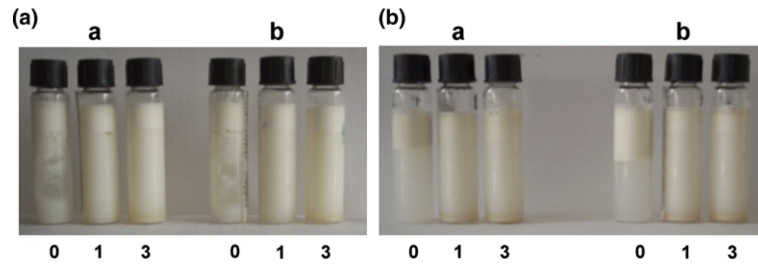


Fig. 2. Creaming behaviour of emulsions prepared with heat-treated SPI–dextran mixtures, after storage for 15 days. (a) pH 6.5; (b) pH 3.8; (a) protein/polysaccharide weight ratio 1/1; (b) protein/polysaccharide weight ratio 1/3. Numbers indicate incubation time in weeks.

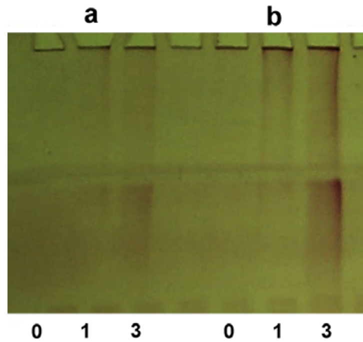


Fig. 3. Upper parts of SDS–PAGE patterns stained for carbohydrate, for heat-treated SPI–dextran (1/1 w/w), absorbed in emulsions: (a) pH 6.5; (b) pH 3.8. Numbers indicate incubation time in weeks.

Table 5
Protein adsorbed per unit interface, $\Gamma_s$ (mg/m$^2$), in o/w emulsions prepared with heat-treated (at 60 °C) SPI or SPI–dextran mixtures, determined one day after preparation

| Sample | pH 6.5 | | | pH 3.8 | | |
|---|---|---|---|---|---|---|
| | Incubation time (weeks) | | | | | |
| | 0 | 1 | 3 | 0 | 1 | 3 |
| SPI | 2.5[a] | 1.8[a] | – | 19.1[a] | 24.9[a] | – |
| SPI–dextran (1/1) | 2.5[a] | 2.1[a] | 5.8[b] | 12.1[a] | 2.6[b] | 4.0[b] |
| SPI–dextran (1/3) | 2.0[a] | 2.3[a] | 2.5[a] | 14.2[a] | 2.5[b] | 2.7[b] |

[a,b] Means for each pH within a row with same superscripts are not significantly different ($p < 0.05$).

approaching each other, it is apparent that they play a secondary role compared to the stability offered to the droplets by the interfacial membrane properties, such as thickness, cohesiveness and rheological properties.

As shown in Table 5, the amount of protein adsorbed per unit interface is influenced by the continuous phase pH and/or the formation, or not, of a protein–polysaccharide hybrid. At pH 3.8, where the protein is not soluble, the molecules are adsorbed as coagulated entities, resulting in increased protein coverage of the interface, something that was also observed in the case of heat-treated lupin protein (Pozani, Doxastakis, & Kiosseoglou, 2002) and which can be attributed to the turbulent conditions prevailing during emulsion homogenization.

Analysis of the SDS electrophoresis zones indicated that the Maillard-type protein–polysaccharide hybrids can effectively adsorb at the emulsion oil-droplet interface, although not to the same extent as found in the initial protein–polysaccharide sample (Table 1), compared to the other constituents of the heat-treated protein–polysaccharide samples (Table 6). It appears, also, that the 11S subunits non-conjugated to dextran adsorbed as effectively as the rest of the protein constituents of the mixture, although one might have expected that they would be adsorbed less effectively due to the lower surface activity compared to 7S proteins (Rivas, 1982).

Table 6
Composition (%)[a] of adsorbed protein in emulsions prepared with SPI–dextran mixtures heat-treated at 60 °C for up to 3 weeks

| Protein constituent | pH 6.5 | | | | | | pH 3.8 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Protein/polysaccharide weight ratio | | | | | | | | | | | |
| | 1/1 | | | 1/3 | | | 1/1 | | | 1/3 | | |
| | Incubation time (weeks) | | | | | | | | | | | |
| | 0 | 1 | 3 | 0 | 1 | 3 | 0 | 1 | 3 | 0 | 1 | 3 |
| Maillard products | 0.0 | 42.8 | 48.5 | 0.0 | 45.1 | 57.9 | 0.0 | 40.4 | 51.4 | 0.0 | 46.9 | 58.3 |
| 7S α′ sub. | 9.9 | 5.0 | 3.7 | 10.6 | 5.4 | 3.2 | 7.6 | 5.5 | 4.1 | 9.0 | 5.5 | 5.2 |
| 7S α sub. | 10.4 | 5.5 | 3.5 | 10.8 | 5.2 | 4.6 | 8.3 | 5.2 | 3.7 | 9.9 | 5.2 | 5.3 |
| 7S β sub. | 12.1 | 7.1 | 8.0 | 11.2 | 6.7 | 5.3 | 13.4 | 10.3 | 6.6 | 12.5 | 8.5 | 8.5 |
| 11S Acidic sub. | 31.9 | 17.8 | 13.7 | 35.8 | 16.6 | 12.4 | 36.9 | 15.1 | 12.3 | 36.4 | 14.9 | 8.5 |
| 11S Basic sub. | 31.2 | 18.5 | 20.1 | 27.1 | 17.2 | 13.8 | 30.6 | 19.2 | 18.8 | 27.6 | 20.3 | 16.5 |
| Other protein constituents | 4.6 | 3.2 | 2.5 | 4.5 | 3.7 | 2.9 | 3.2 | 4.2 | 3.0 | 4.5 | 3.0 | 2.8 |

[a] C.V.%: 8.4.

## 4. Conclusions

Incubation of SPI–dextran mixtures for 1 week resulted in the improvement of emulsifying ability at pH 3.8 but not at pH 6.5 while the stability of the system against creaming, at both of these pH values, increased.

The protein–polysaccharide hybrids are adsorbed at the interface together with unreacted protein constituents of SPI due to their protein moiety enhancing steric stabilization forces operating between neighboring oil droplets.

The adsorption of the conjugate at the interface is satisfactory although, due to its bulky phase, it is not as great as that of other non-reacting SPI protein constituents.

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