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Effects of Soy Protein on Physical and Rheological Properties of Wheat Starch

It is necessary to understand the interaction phenomena between proteins and polysaccharides for the development of starch-based products with better physical and sensory properties. A simplified model system was chosen to study the influence of soy protein on physical and rheological properties of wheat starch and the possible interactions between them. Thermal and pasting behaviors of the slurries and texture properties, water retention capacity and ultra structure of soy protein-wheat starch gels were analyzed. While soy protein isolate increased the viscosity of starch suspension during and after heating, gels with soy protein presented a weaker structure than wheat starch gels. Results suggested association between leached out material and swollen granule surface of starch with soy protein. Scanning electron microscopy reflected these changes in the gel ultrastructure.

Keywords: Starch; Soy protein isolate; Gel

1 Introduction

Childhood malnutrition, in the form of protein-energy malnutrition, is the most common deficiency disease in the world, especially in developing countries. This is related to poor food quality, insufficient food intake and infections [1]. Legume proteins are major components in the diet of food-producing animals and are increasingly important in human nutrition. Soybean is the most important legume in relation to total world crop production and the most frequently used because of its high protein content and relatively low price [2].

The addition of soy ingredients to traditional foods can improve their protein quality. Consumption of soy foods is increasing because of reported beneficial effects on nutrition and health, such as lowering of plasma cholesterol, prevention of cancer, diabetes and obesity, and protection against bowel and kidney diseases [3].

Nunes et al. [4] studied the replacement of milk by lupine, pea and soy proteins, in combination with κ -carrageenan, gellan or xanthan gum, on the rheological properties of starch-gelled desserts. They reported that the milk/carrageenan gel showed higher firmness than the vegetable ones, which seemed to be related with specific interac-

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tion between carrageenan and caseins. Of the vegetable products, the gel that had the highest value of firmness was the sov/carrageenan mixture. Gels studied under steady shear conditions showed similar flow curves, exhibiting a strong shear-thinning behavior. Soy and pea gels had higher viscosity values than milk and lupine gels. These authors, considering both texture and rheological measurements, found that vegetable proteins and k-carrageenan or gellan gum would be good systems to develop gelled desserts, in which animal proteins are fully replaced. Lim and Narsimhan [5] investigated the pasting and rheological behavior of slurries made from commercial sov proteins and modified maize starches. Inclusion of soy protein in the system increased pasting temperature and overall viscosity of starch/HFCS (high fructose corn syrup) paste and this trend was related to the increase in the concentration of solid contents, and to the self-aggregation of soy globulins. They developed some formulations with rheological properties closer to the commercial puddings tested.

Despite these results, the development of products with better physical and sensory properties has become necessary. The understanding of the interaction phenomena of proteins with the polysaccharides is imperative to reach this objective. Furthermore, a better comprehension of the physicochemical changes in the soy/starch system may lead to acceptable formulations. In this paper, we chose a simplified model system to study ingredient interactions. Once the mean interactions are understood, the study of incorporation of soy protein on real gelled-starch desserts will be extended.



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The objective of the research presented in this paper was to study the influence of soy protein on physical and rheological properties of wheat starch and the soy protein-starch interactions.

2 Materials and Methods

2.1 Materials

Commercial soy protein isolate (moisture content: $6.1 \pm 0.0 \text{ g/100}$ g, protein content: $85.5 \pm 0.0 \text{ g/100}$ g, wet basis) (SPI) Samprosoy 90 HI was obtained from Solae (Sao Paulo, Brazil); 90% of SPI particles pass through a 100 mesh sieve. Native wheat starch (moisture content: $11.4 \pm 0.2 \text{ g/100}$ g, protein content: $0.18 \pm 0.01 \text{ g/100}$ g, wet basis) was purchased from Sigma Chemical (S5127) (St. Louis, MO, USA); 95% of starch particles pass through a 120 mesh sieve.

2.2 Differential scanning calorimetry (DSC)

Analyses were performed in a DSC823e calorimeter (Mettler Toledo, Schwerzenbach, Switzerland), The equipment was calibrated with indium and zinc, and empty, sealed but pierced, aluminum pans were used for reference. Soy protein isolate was added to wheat starch (10, 30 and 50% w/w of starch). The starch and proteinstarch dispersions (10:90 starch: water ratio) were prepared by mixing solids and distilled water. These slurries were allowed to equilibrate in a closed flask for 1 h at room temperature with continuous stirring. Aliguots of 14-16 mg were taken from the flask with continuous stirring and were weighed in 40 µL aluminum DSC pans. Pans were hermetically sealed to avoid sample dehydration and scanned at 5°C/min from 25 to 120°C. Onset temperature (T_{o}), peak temperature (T_{o}), range temperature (ΔT) , as well as the heat of phase transition $(\Delta H, \text{ in joules})$ per gram of dry matter) of starch gelatinization were determined using STARe software provided by the manufacturer. All measurements were done in triplicate.

2.3 Pasting properties

Pasting properties of starch and starch-protein mixtures were determined using a Micro-Viscoamylograph (Brabender, Duisburg, Germany). Soy protein isolate was added to 5 g wheat starch (10, 30 and 50% w/w of starch). Starch and starch-soy protein mixtures were dispersed in 95 mL of distilled water. The slurries were directly placed in a stainless steel measuring bowl and then heated from 30°C to 95°C, held for 5 min at 95°C, and cooled to 50°C, held for 5 min at 95°C. Heating and cool-

ing rates were 3°C/min. The parameters recorded were initial pasting temperature (PT), temperature at maximum viscosity (TMV), peak viscosity (PV), hot paste viscosity (HPV), final or cool paste viscosity (CPV), breakdown (BD) and setback (SB). All experiments were run in duplicate and the coefficient of variation of viscosity properties was less than 5% at any point along the curve.

2.4 Preparation of gels

Soy protein isolate was added to 10 g wheat starch (10, 30 and 50% w/w of starch). Starch (ST) and starch-protein mixtures were dispersed in distilled water (90 mL) in a flask. In order to prevent microbial spoilage of the stored samples, sodium azide (0.1%, w/w) was added to the suspensions. The mixtures were allowed to equilibrate for 20 min at room temperature with continuous stirring. Gelatinization was carried out by immersing the hermetically closed flask in a water bath at 95°C for 30 min. Manual agitation was used to avoid heterogeneity in the gelled system. The suspension was filled while hot (90°C) in polypropylene tubes, 30 mm diameter, cooled to room temperature (~25°C) and kept for 4 h at that temperature. The samples were stored at 4°C before analyzing the gel properties.

2.5 Textural analysis

Large deformation measurements were performed in a TA-XT2 Texturometer (Stable Microsystems, Surrey, Great Britain) equipped with a stainless steel cylindrical probe (50 mm diameter). Hot mixtures (prepared as previously described) were poured into polypropylene tubes (30 mm diameter) and cooled to room temperature and kept for 4 h at that temperature. The cooled samples were stored at 4°C before analyzing. At 0, 7 and 15 days, the gels were cut into discs of 1 cm height and analyzed. Each gel disc was subjected to a double cycle of compression (TPA procedure), under the following conditions: crosshead speed, 0.5 mm/s and maximum deformation, 30%. Compression cycles were separated for 10 s. The cylindrical probe covered the total surface area of the gel disc during compression. The texture profile parameters were determined using the Texture Expert 1.22 (Stable Microsystems, Surrey, UK). Gel firmness, chewiness and gumminess were calculated with a force-distance graph. Six gel discs were analyzed for each duplicated gel and average values were reported.

2.6 Syneresis

Syneresis was measured in a centrifugation test using a Beckman J2-MI centrifuge (Beckman Instruments, Full-

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erton, USA). Starch and starch-soy protein dispersions (prepared as described previously) (~15 g) were placed into 50 mL centrifuge tubes while they were hot and stored at 4°C for 28 days. After storage, the gels were tempered at 20°C (2 h) and were centrifuged at $1500 \times g$ for 15 min at 20°C. After centrifugation the free water was separated, weighed and expressed as percentage of the total present in the gel. Measurements were the mean of three repetitions for each duplicated gel.

2.7 Scanning electron microscopy (SEM)

The structural changes of starch-protein gels were observed using SEM. For this assay, some portions of gels produced for texture analysis were separated. Small pieces ($\sim 5 \text{ mm}^3$) were cut with a razor blade, fixed in glutaraldehyde (1:30) and embedded in a graded acetone series (25, 50, 75 and 80%) for 20 min at each gradation, then embedded in 100% acetone at three consecutive 20 min intervals to ensure full dehydration. The samples were then critical point dried. Critical point drying allows acetone removal in CO2 without surface tension force that may distort the sample. The dehydrated samples were coated with gold particles for 4 min. The images were taken using a Jeol 35 CF (Jeol Ltd, Tokyo, Japan) scanning electron microscopy at 6 kV acceleration voltage. The micrographs were taken by using different magnification (5000 \times , 7500 \times and 10,000 \times).

2.8 Protein solubility

Protein solubility was assessed from starch-soy protein gels and heated-SPI (containing no starch) dispersions. Soy protein isolate (10, 30 and 50% w/w of starch) was added to 5% wheat starch. SPI suspensions were formulated keeping the same protein: water ratio as in starch-SPI gel formulations. Starch-SPI and SPI suspensions were allowed to equilibrate for 20 min at room temperature with continuous stirring. Heating was carried out by immersing the hermetically closed flasks in a water bath at 95°C for 30 min. Manual agitation was used to avoid heterogeneity in the system. Suspensions were filled while hot (90°C) in 30 mm diameter polypropylene tubes, cooled to room temperature (~25°C) and kept 4 h at that temperature. The samples were stored at 4°C before analyzing the protein solubility.

At different times of storage, aliquots of the suspensions (3.0 g) were mixed and vortexed for 2 h with (4 mL) 100 mM phosphate buffer (pH 8.0). The slurries were centrifuged for 15 min at $2000 \times g$ and 25° C and the supernatant separated. The protein content of the supernatants was determined using the Bradford protein assay proce-

dure. Measurements were the mean of two repetitions for each duplicated dispersion.

2.9 Statistical analysis

The data obtained were statistically treated using analysis of variance while the means were compared by the LSD Fisher test at a significance level of 0.05 using, in both cases, the INFOSTAT statistical software (Facultad de Ciencias Agropecuarias, UNC, Córdoba, Argentina).

3 Results and Discussion

3.1 Differential scanning calorimetry

SPI suspensions did not exhibit a peak of denaturation in the DSC thermogram from 30 to 125°C (results not showed). In the same way, SPI showed high values of Nitrogen Solubility Index (82.1±1.9%) and did not presented urease activity [6]. These results indicated an unfolded denatured protein state due to the production procedure. The thermograms obtained from starch and protein-starch suspensions presented an endotherm corresponding to the gelatinization of the starch. Onset and final temperatures ranged between 56°C and 58°C and between 65°C and 67°C, respectively, and the absorbed energy range from 8.9 and 11.9 J/g of starch (Tab. 1). Onset and peak temperatures increased significantly with protein addition beginning at 30% of SPI. The increase in peak temperatures could be related to the interaction between material leached out of the granules and protein and/or between surface granules and protein. Protein addition did not have a significant effect (p > 0.05) on the gelatinization temperature range (ΔT) suggesting similar crystallite stability. Among SPI-starch mixtures, increasing the SPI content resulted in lower heat of gela-

Tab. 1. Effect of SPI addition on the onset temperature (T_{o}) , peak temperature (T_{p}) , temperature range (ΔT) , and heat of phase transition (ΔH) of starch suspensions (starch: water, 10:90).

Sample	7₀ [°C]	T _p [°C]	∆ 7 [° C]	ΔH^1 [J/g]	Δ H ² [J/g]
ST	56.96a	61.21a	19.51a	-8.92a	-8.92c
ST-SPI10	57.05a	61.27a	21.31a	-8.79a	-9.77bc
ST-SPI30	57.49b	61.91b	20.91a	-7.10b	-10.14b
ST-SPI50	57.94c	62.42c	20.20a	-5.96c	-11.91a

¹ Enthalpy of starch suspensions (expressed in J/g total solids).

² Enthalpy of starch suspensions (expressed in J/g dry starch).

Values followed by the same letter are not significantly different (P > 0.05).

tinization (ΔH). The ΔH for the mixture (SPI + starch, dry basis) was then converted to that for starch as listed in Tab. 1, because only starch exhibited an endothermic peak. The data showed that ΔH for starch increased steadily and significantly with protein addition. Similar results were reported by *Li* et al. [7], who studied the properties of maize starch/soy protein concentrate composites during heating. These authors indicated that the presence of soy protein restricted the swelling and gelatinization of starch, thus a higher temperature and more energy was required for gelatinization.

3.2 Pasting behavior

In order to assess the effect of soy protein on starch viscosity behavior, the pasting properties were studied. The initial pasting temperatures (Micro-Amylograph) (Tab. 2) of starch suspensions were higher than their corresponding T_o (DSC) (Tab. 1) and even higher than the conclusion temperatures of gelatinization, suggesting that the initial increase in viscosity occurred when the starch granules were completely melted.

Addition of SPI changed the pasting properties of wheat starch (Tab. 2). The initial pasting temperature (PT) decreased gradually, while the temperature at the maximum viscosity did not change with protein addition. This trend was opposed to the behavior of the transition temperatures (T_o and T_p) measured by DSC.

Biliaderis [8] stated that the gelatinization process, measured by DSC, represented net thermodynamic quantities of different events: granule swelling and crystallization (endothermic), and hydration and recrystallization (exothermic), and contributions from the amorphous regions. Later, *Cooke* and *Gidley* [9] suggested that the gelatinization enthalpy primarily reflected the loss of molecular (double-helical) order. *Tester* and *Morrison* [10]

Tab. 2. Effect of SPI addition on starch pasting behavior.

	ST	ST-SPI10	ST-SPI30	ST-SPI50
PT [°C]	90.8	87.7	81.5	74.2
TMV [°C]	95.6	94.3	95.2	95.0
PV [BU]	90	99	123	168
HPV [BU]	84	93	102	117
CPV [BU]	177	186	195	219
BD [BU]	6	6	21	51
SB [BU]	93	93	93	102

PT= initial pasting temperature, TMV= temperature at the maximum viscosity, PT =, PV = peak viscosity, CPV = cool paste viscosity, HPV = hot peak viscosity, BD = break-down (PV-HPV), SB = setback (CPV-HPV), STABR = stability ratio (HPV/PV), BU = Brabender unit.

reported that swelling started at a temperature corresponding to the onset temperature in DSC measurements, but it continued at higher temperature. Thus, the onset of the DSC gelatinization transition was expected to depend on a complex interplay of factors, among which water transport into the granules could be considered to play a very important role [11]. On the other hand, during pasting, an increase in viscosity was not measured until swelling and leaching have proceeded to some extent. Shear force caused rupture of the starch granules, which in turn influenced the leaching from the granules. It was thus easily understood that the rheological properties of a paste would depend on the procedure. Similar differences between pasting temperature and onset temperature recorded by DSC working with normal and waxy wheat starch were reported by Mira et al. [11]. These authors suggested that the differences inherent to the experimental techniques, such as sample size, mechanical shear, and starch concentration; could influence the gelatinization temperature values.

Protein molecules could affect the gelatinization process in different ways depending on their ability to retain water and their interaction capacity with the starch molecules and surface granules. The decrease of PT, as a consequence of protein addition, also may be attributed to an increase in the effective concentration of starch in the continuous phase, because the hydration and solubilization of soy protein progress with temperature increment.

The pasting behavior was steadily affected by SPI addition. SPI increased the paste viscosity of wheat starch during the heating and cooling period, as is shown by PV and CPV values (Tab. 2). Eliasson and Gudmundsson [12] described how during heating, at the same time that water is absorbed, material was leached out from the starch granules. This material was largely amylose, although amylopectin and intermediate material (less branched than amylopectin) were present, the amount depended on the starch, the shearing force and the pasting conditions. These researchers also described the gelatinized starch suspension as a composite material composed of a dispersed phase (starch granules) in a continuous polymer solution (amylose/amylopectin). The rheological properties of such system depend on properties of the components themselves as well as their ratio and the interaction between them.

Proteins contain many hydrophilic groups (such as -COOH, $-NH_2$, -OH, and -SH) all of which are capable of forming crosslinks with starch. These crosslinks may be responsible for their higher paste viscosity as compared to a starch paste [13]. The pH of slurries and gels was determined. Starch, ST-SPI10, ST-SPI30 and ST-SPI50 slurries presented pH values of 6.00, 6.37, 6.12 and 6.09,

respectively; and starch, ST-SPI10, ST-SPI30 and ST-SPI50 gels had pH values of 5.34, 6.23, 6.28 and 6.22, respectively. These results showed that soy protein, under these conditions (pH values higher than isoelectric point), presented collective negative charge before and after gel production, leading to a decreased strength of the protein-protein interactions and, possibly, to a reinforcement of protein-amylose and protein-amylopectin interactions. Likewise, *Lim* and *Narsimhan* [5] showed that soy proteins increased pasting temperature and overall viscosity of soy protein/starch/HFCS pastes. They suggested that this trend could be primarily due to an increase in the concentration of solid contents resulting from the addition of soy proteins, and/or through selfaggregation of soy globulins.

Besides, *Eliasson* and *Gudmundsson* [12] reported that the rheological behavior of a gelatinized starch suspension also depended on the adhesion between the dispersed phase and the matrix. It is also possible that the starch-protein interactions affected adhesion between phases.

The breakdown in viscosity coincided with protein addition beginning at 30% of SPI. High values of breakdown were associated with high peak viscosities. The setback of wheat starch was increased only at the maximum addition level of SPI. For starch paste, the increase in paste viscosity during cooling indicated the association of starch molecules, particularly amylose. Two mechanisms were proposed to explain the gelation of amylose: phase separation between polymer-rich and polymer-deficient phases, with a subsequent development of crystalline zones in the polymer-rich region [14] and amylose aggregation due to crosslinking of long chains [15]. The increment in the setback value could have resulted from the increase in the concentration of solid contents resulting from the addition of soy proteins.

3.3 Textural analysis

Fig. 1 shows gel firmness as measured by TPA analysis. The addition of SPI to starch produced significantly softer gels at all testing times. SPI-starch gels had similar values of firmness at the beginning of the storage period but ST-SPI10 gels were harder than ST-SPI30 and ST-SPI50 during the storage period, indicating some influence of soy protein on the amylopectin retrogradation process.

It has to be borne in mind that when the gelatinized starch cools, retrogradation of amylose results in an increase in viscosity until a gel is formed. The gel is essentially a three-dimensional network of intertwined leached out material incorporating dispersed swollen and rupture

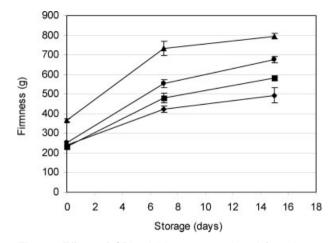


Fig. 1. Effect of SPI addition on starch gel (starch-to-water ratio 10:90) firmness during storage at 4°C. Starch (\blacktriangle), ST-SPI10 (\bigcirc), ST-SPI30 (\diamondsuit) and ST-SPI50 (\blacksquare). Error bars show standard deviation.

granules. Gel firmness will increase with time as a consequence of starch change from an amorphous to a more orderly and crystalline state. It is accepted that the shortterm development of gel structure results from amylose crystallization, and long-term reordering of amylopectin is a much slower process involving recrystallization of the outer branches of this polymer [16].

Our research confirmed that interrupting the interchain association between amylose molecules by inclusion of chain segments of sufficient length would lead to a weakened gel network structure [17].

On the other hand, it is known that starch granules influence the rheological properties of the starch gels due to their phase volume and their deformability, but also due to the adhesion between the filler phase and the matrix [12]. Being ionic in nature, soy proteins may interact readily with amylose and exposed branches of amylopectin through non-covalent bonding, especially hydrogen bonds. The amylose- and starch granules-protein interactions could contribute to gel matrix weakening. Similar trends have been reported by other authors [18], who showed that whey protein isolate (WPI) weakened the gel structure of wheat starch at 30°C. These authors have suggested that the WPI acted as inactive filler decreasing the association of hydrogen bonds in the gel matrix and diluted gel strength of the starch fraction.

Fig. 2 shows the effect of soy protein addition on the chewiness (a) and gumminess (b) of the gels. Chewiness is related to the energy required to disintegrate a solid food to a state ready for swallowing while gumminess is related to the same energy but to disintegrate a semi-solid food [19]. Starch gels are soft material, but ST-SPI50

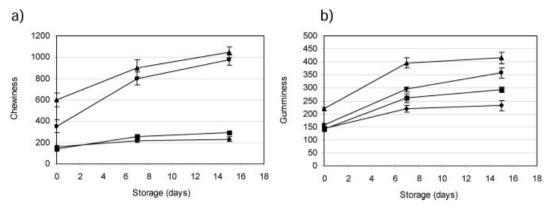


Fig. 2. Effect of SPI addition on starch gel (starch-to-water ratio 10:90) chewiness during storage at 4°C. Starch (▲), ST-SPI10 (●), ST-SPI30 (♦) and ST-SPI50 (■). Error bars show standard deviation.

gels are much softer than starch gels, almost semi-solid materials.

Both gumminess and chewiness were analyzed to better describe gel properties. The addition of SPI to starch produced gels that had lower chewiness and gumminess. Gel gumminess showed a similar trend to gel firmness but gel chewiness presented some differences. ST-SPI30 and 50 presented lower increment of chewiness with the storage time than starch and ST-SPI10 gels, suggesting high gel weakness over the storage time.

3.4 Syneresis

Separation of water from starch gels or starch-containing products is usually viewed unfavorable, because it is considered to produce product deterioration. In our research, water separation of the gels (5% w/w starch) incremented with the storage time at 4°C (Fig. 3). *Zheng* and *Sosulski* [20] showed that the reorganization of starch molecules or retrogradation of starch-based systems during cold storage resulted in a release of water. *Perera* and *Hoover* [21] pointed out that the increase in syneresis during storage could be attributed to the interaction between leached amylose and amylopectin chains, which lead to the development of junction zones.

Addition of soy protein steadily increased the syneresis of the samples at each storage time. These results were not expected due to the high water retention capacity of soy protein. Soy protein addition to wheat starch should result in higher water retention as compaired with a system formed by the same amount of starch without soy protein addition, assuming the contribution of the individual macromolecules. The measured values indicated that interaction occurred between the proteins and the starch, which could be responsible for the low water-binding capacity. The water retention properties of the composite

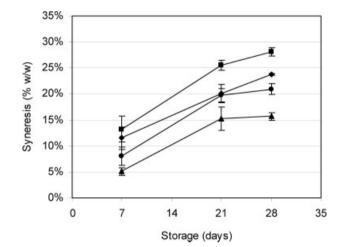


Fig. 3. Syneresis of starch-SPI pastes (starch-to-water ratio 5:95) as a function of the storage time at 4°C. Starch (▲), ST-SPi10 (●), ST-SPI30 (♦) and ST-SPI50 (■). Vertical bar represents standard deviation.

network prevailed against water retention properties of the individual macromolecules. At the same time, because of the water-absorbing capacity of soy proteins, they may have competed for water with starch during pasting. After the storage period, this protein-retained water could be easily separated in comparison with the starch-retained water.

3.5 Scanning electron microscopy

Scanning electron micrographs of starch gels with and without soy proteins are shown in Fig. 4. The gel structure could be described as a continuous matrix, the leached out material, enveloping the swollen and fragmented starch granules (G). Starch granules are shown like empty balloons, which seemed acceptable due to the drying

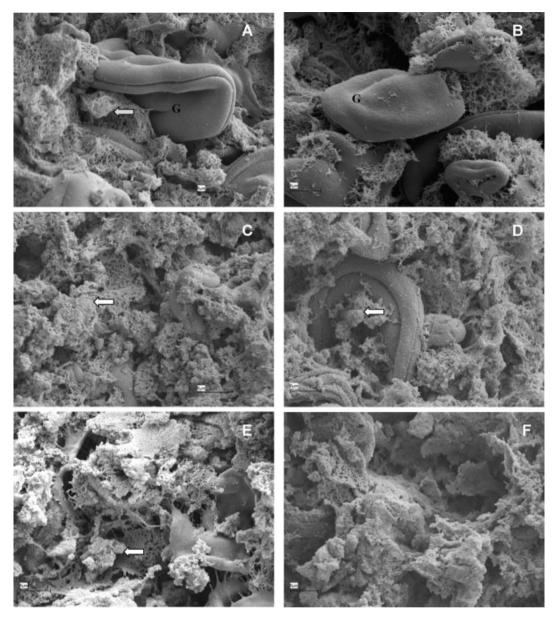


Fig. 4. Scanning electron microscopy of starch gels. Non-stored (A) and 15 days stored (B) starch; non-stored (C) and 15 days stored (D) ST-SPI-30; and non-stored (E) and 15 days stored (F) STSPI50.

process necessary to sample preparation. The granule surfaces were smoother in starch gels without SPI than in SPI-starch gels, indicating that soy proteins could be attached to the granule surface. The continuous phase was more compact in SPI-starch gels than in starch gels. Fig. 5 shows clearly the effects of soy protein addition on gel structure. Starch gels (without SPI) showed a more open and regular continuous phase (A). ST-SPI30 gels (B) presented a more compact continuous phase, probably composed of a mixture of leached out material and soy proteins. ST-SPI50 gels (C) showed globular and compact structures adhering to the granule surface and the continuous phase. The structures are probably composed of largely soy proteins. These results confirmed the association between leached out material and swollen granule surface with soy protein, whereby starch gels were softer than SPI-starch gels.

3.6 Protein solubility

To obtain information on starch and soy protein interaction, starch-SPI gels and SPI suspensions (not containing starch) were produced as described in Materials and



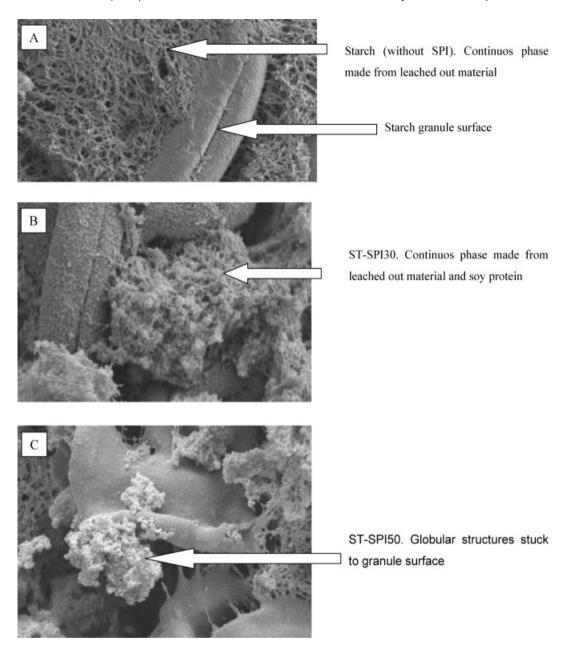


Fig. 5. Scanning electron microscopy of starch gels. A) Starch without SPI; B) ST-SPI130; C) ST-SPI150.

Methods. At different times of storage, aliquots of the suspensions were mixed with phosphate buffer and the protein content of the supernatants was determined. Fig. 6 shows the protein content of starch-SPI (a) and SPI (b) suspensions at 0, 7 and 14 days of storing at 4°C. Supernatants from starch-SPI gels had higher protein content than supernatants from SPI suspensions after gel production. At 7 and 14 days of storage, supernatants from starch-SPI gels and SPI suspensions had similar protein contents, except when SPI was added at the

highest proportion. These results indicated that after gel production, soy proteins interacted with leached out starch material, hence decreasing protein aggregation as consequence of heating and increasing protein solubility in the buffer solution tested. SPI suspension ageing decreased protein solubility possibly due to additional protein aggregation. Starch-SPI gel ageing decreased protein solubility in a greater proportion than SPI suspension, suggesting that the reorganization of starch molecules had affected protein solubility. 622 P. D. Ribotta et al.

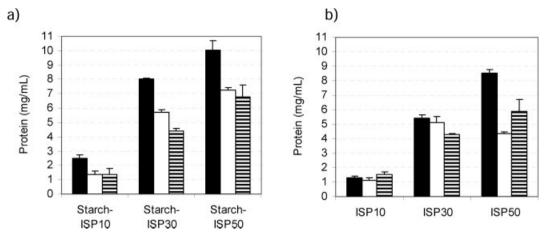


Fig. 6. Protein solubility from starch-SPI (a) and SPI (b) suspensions at 0 (black), 7 (white) and 14 (lack lines) days of storage at 4°C.

4 Conclusions

Soy protein isolates increase the viscosity of starch suspensions during and after heating and modify the gelatinization and pasting temperatures of starch.

Gel texture and syneresis values demonstrate that gels with SPI present a weaker structure than starch pastes and gels.

It is suggested that these changes are related to the association between leached out material and the swollen granule surface with soy proteins. Scanning electron microscopy reflects these changes in the gel microstructure.

Clearly, the gelation ability of wheat starch and soy proteins could present an interesting alternative to gelled milk-starch products. The present study provides insight into the interaction phenomena of these proteins with the polysaccharides. However, a more thorough research on the related mechanism of starch gelation, such as additive addition and different pH conditions, should be carried out.

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References

- M. Onis, M. Blossner: WHO global database on child growth and malnutrition. Geneva: Programme of Nutrition, World Health Organization, 1997.
- [2] Y. Gupta: Nutritive value of soybean. *Int. J. Tropical Agric.* **1987**, 5, 247–279.
- [3] M. Friedman, D. Brandon: Nutritional and health benefits of soy proteins. J. Agric. Food Chem. 2001, 49, 1069–1086.
- [4] M. C. Nunes, P. Batista, A. Raymundo, M. M. Alves, I. Sousa: Vegetable proteins and milk puddings. *Col Surf B: Biointerfaces* 2003, 31, 21–29.
- [5] H. S. Lim, G. Narsimhan: Pasting and rheological behavior of soy protein-based pudding. *LWT- Food Sci. Tech.* 2006, 39, 343–349.
- [6] P. D. Ribotta, S. A. Arnulphi, A. E. León, M. C. Añón: Effect of soybean addition on the rheological properties and breadmaking quality of wheat flour. *J. Sci. Food Agric.* 2005, 85, 1889–1896.
- [7] J.-Y. Li, A.-I. Yeh, K.-L. Fan: Gelation characteristics and morphology of corn starch/soy protein concentrate composites during heating. *J. Food Eng.* 2007, 78, 1240–1247.
- [8] C. Biliaderis: Thermal analysis of food carbohydrates, in *Thermal Analysis of Foods* (Eds. V. Harwalkar, C.-Y. Ma) Elseiver, New York, **1990**.
- [9] D. Cooke, M. J. Gidley: Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition. *Carbohydr. Res.* **1992**, 227, 103–112
- [10] R. F. Tester, W. R. Morrison: Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. *Cereal Chem.* **1990**, 67, 551–557.
- [11] I. Mira, K. Persson, V. K. Villwock: On the effect of surface active agents and their structure on the temperatureinduced changes of normal and waxy wheat starch in aqueous suspension. Part I. Pasting and calorimetric studies. *Carbohydr. Polym.* **2007**, 68, 665–678.
- [12] A. Eliasson, M. Gudmundsson: Starch: Physicochemical and functional aspects, in *Carbohydrates in Food* (Ed. A. Eliasson) Marcel Dekker, New York, **1996**.

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- [13] P. K. Goel, R. S. Singhal, P. R. Kulkarni: Studies on interactions of corn starch with casein and casein hydrolysates. *Food Chem.* **1999**, *64*, 383–389.
- [14] M. J. Miles, V. J. Morris, P. D. Orford, S. G. Ring: The roles of amylose and amylopectin in the retrogradation of starch. *Carbohydr. Res.* **1985**, *135*, 271–281.
- [15] M. J. Gidley: Molecular mechanisms underlying amylose aggregation and gelation. *Macromolecules* **1989**, *22*, 351– 358.
- [16] A. A. Karim, M. H. Norziah, C. C. Seow: Methods for the study of starch retrogradation. *Food Chem.* 2000, 71, 9–36.
- [17] C. G. Biliaderis, J. Zawistowski: Viscoelastic behavior of aging starch gels: effects of concentration, temperature, and starch hydrolysates on network properties. *Cereal Chem.* **1990**, 67, 240–246.

Effects of Soy Protein on Properties of Wheat Starch 623

- [18] H. Yang, J. Irudayaraj, S. Otgonchimeg, M. Walsh: Rheological study of the starch and dairy ingredient-based food systems. *Food Chem.* **2004**, *86*, 571–578.
- [19] SMS Stable Micro Systems: Available in http://www. stablemicrosystems.com, captured in December 2006.
- [20] G. H. Zheng, F. W. Sosulski: Determination of water separation from cooked starch and flour pastes after refrigeration and freeze-thaw. J. Food Sci. **1998**, 63, 134–139.
- [21] C. Perera, R. Hoover: Influence of hydroxypropylation on the retrogradation properties of native, defatted and heat – moisture treated potato starches. *Food Chem.* **1999**, *64*, 361–375.

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