Influences of Physicochemical Characteristics of Rice Flour and Cassava Starch on the Gelation of Calcium-Induced Egg Albumen-Flour Composite

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ABSTRACT

Mechanical properties of calcium-induced composite gel made of egg albumen (EA) containing 15% protein (w/v) and 30% of rice flour (RF), cassava starch (CS) or a mixture of RF and CS at the ratio of 1:1, added with 0-70 mM calcium lactate and 1% of i-carrageenan were characterized. The two-phase exponential decay model suggests that the calcium lactate concentration did not have significant effect on the stress decay mechanism of the flour-protein composite gel ($p \geq 0.05$). However, the type of flour filler regulated the stress decay behavior ($p < 0.05$). This was due to the high content of flour to protein ratio in the composite. Such protein to starch ratio led to the close-packing composite structure, where the swollen starch granules packed densely within the continuous EA protein matrix. The EA-CS composite gel relaxed faster and to a much higher extent than the EA-RF gel. This was likely to be due to the high capacity of CS to absorb water, which weakened the strength of the granule after the composite structure was heated during structure-forming process. In addition to the lower water absorption ability of RF compared with CS ($p < 0.05$), light micrograph revealed that the reinforcement mechanisms of RF could be due to the higher number of granular particles per unit volume of the RF in the composite gel and the aggregation of the swollen and deformed amylose-rich fraction of the RF after heat-treatment. The aggregation of CS granules and remnants was not evident in the heated EA-CS composites.

Key words: cassava, composite, egg albumen, gel, rice

INTRODUCTION

Starches and proteins are the main ingredients in food formulations. Both biopolymers undergo molecular and microstructural changes during cooking and cooling processes. Mechanism of starch reinforcement in a protein-starch composite matrix relies upon mass fraction of both macromolecules, water availability, gelation temperature of continuous matrix, the volume fraction of the separated phase or filler, as well as the mechanical strength of the fillers in relation to the continuous matrix (McClements *et al.*, 1993). Generally, the reinforcement could be obtained with increasing volume fraction of the filler (Richardson *et al.*, 1981; Ross-Murphy and Todd, 1983).

It was recently reported that the viscoelasticity of protein-starch composite structure could be enhanced provided that the protein matrix was formed prior to the starch gelatinization (Wongsasulak *et al.*, 2004) by using a salt-induced gelation method of the globular

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proteins described by Barbut and Foegeding (1993) and Doi (1993). The use of two-stage gelation of protein matrix introduced by Wongsasulak *et al*. (2004) could avoid phase inversion of the continuous protein phase to gelatinized starch phase occurred when the starch to protein ratio was higher than 3 : 10 reported by the other investigators (Aguilera and Rojas, 1996; 1997; Aguilera and Baffico, 1997).

The salt-induced gelation and film formation of the egg albumen (EA) and cassava starch (CS) has been previously demonstrated as a potential carrier and coating for the oil-soluble compound such as paprika oleoresin by Wongsasulak *et al*. (2006). The release characteristics of the core material from the starch-protein composite matrix are mainly dependent on the microstructure of the composite, which were influenced by the composition, types and concentration of salt, temperature and water availability during its applications.

Apart from the salt types and their concentrations, the source of starch also played an important role in determining the mechanical properties of the protein-starch composite gel (Hongsprabhas and Dit-udom-po, 2006). The EA-rice flour (RF) composite gels were reported to be more rigid but had lower extensibility than the EA-CS composite gel. It was shown that the mechanical properties of the EA-RF, unlike those of the EA-CS, were dependent on the mechanical properties of the continuous protein phase although the starch to protein ratio was as high as 2:1. We hypothesized that apart from the volume fraction of the filler, the structural reinforcement of the composite was also relied on the mechanical strength of the filler.

This study was aimed to further investigated the roles of starch granular characteristics on the stress transmitting mechanisms of the heated calcium-induced EA-flour composite gels. A better understanding on the influences of the separated starch phase on the mechanical properties of the composite gel could help designing the mechanical properties of the composite structure containing high level of starch.

MATERIALS AND METHODS

Dried egg albumen (EA) powder (High gel type, SA Igreca, France) was obtained from Winner Group Enterprise Ltd., Thailand. The powder had a protein content of 86.25% (dried weight basis) determined by Kjeldahl analytical method using the N factor of 6.25 (AOAC, 2000). Food grade rice flour (RF, 10.28% moisture content, 7.59% protein, Jade Leaf Brand, Bangkok Interfood Co. Ltd., Bangkok, Thailand) and cassava starch (CS, 12.17% moisture content and no protein detected by Kjeldahl method, Jade Leaf Brand, Bangkok Interfood Co. Ltd., Bangkok, Thailand) were used in this study. The ι-carrageenan (Marcel Carrageenan, Quezon City, Philippines) was kindly supplied by Behn Meyer and Co. (T) Ltd., Bangkok, Thailand. Reagent grade of calcium lactate (Fluka, Fluka Chemie AG, Switzerland) was used in entire study.

Thermal properties of food grade rice flour and cassava starch

A Pyris 1 DSC (Perkin Elmer, USA) was used in this study to characterize thermal properties of food grade RF and CS. Flour suspensions of 15 or 30% (w/w) were prepared in distilled water and pipetted into the stainless steel pans, which were later hermetically sealed. The samples were heated at a rate of 12.5 °C/min from 30 to 95 °C to determine the transition temperature and enthalpy of gelatinization. The heating rate used was similar to the rate used in the Rapid Viscoamylography previously reported (Hongsprabhas and Dit-udom-p, 2006).

Effect of flour filler on stress relaxation characteristics of the protein-starch composite gel

The EA suspension (pH 7.6) containing 15 % (w/v) of protein was prepared in distilled water, pre-heated at 55 °C for 5 min, cooled to room temperature (30 °C) and added with RF, RF+CS (1:1) or CS, i-carrageenan and calcium lactate solution to obtain the composite suspension containing final protein concentration of 15% (w/v), flour filler 30% (w/v), i-carrageenan 1% (w/v) in the presence of calcium lactate 0, 10, 30, 50 or 70 mM using method previously described (Hongsprabhas and Dit-udom-po, 2006). The suspensions were poured into polycarbonate tubes with the inner diameter of 20 mm. The tubes were placed in a water bath where the temperature was raised to 80 °C and held at that temperature for 30 min to form gel. All gels were cooled to room temperature (30 °C) for 2 h prior to analyses.

The cylindrical gel sections (20 mm diameter × 10 mm long) were compressed for 20% deformation between a lubricated stationary bottom plate and a moving upper plate with a crosshead speed of 100 mm/min using a Lloyde Texture Analyzer (Series 500, Fareham, UK) for 300 s. The *compressive stress* at time *t* (σ_t) were calculated as follow:

$$\sigma_t = \frac{F_t(L - \Delta L)}{\pi_r{}^2 L}$$

where F_t is the compressive force at time *t*, *L* is the original sample length, ΔL is the corresponding deformation, and *r* is the original radius (Tang *et al.*, 1994). The stress relaxation characteristics were determined as % relaxation which was calculated from the difference between *initial stress (σ_0)* and *residual stress* at 300 s *(σ_{300})* compared with the σ_0 as follow:

$$\% \,\mathrm{relaxation} = \frac{\sigma_0 - \sigma_{300}}{\sigma_0} * 100$$

The stress relaxation data of the EA-flour composite matrix were fitted to the two-phase exponential decay model by a nonlinear regression, using the Grafit software package (Leatherbarrows, 1992) as follows :

$$\sigma t = a * \exp^{(-K_1 * t)} + b * \exp^{(-K_2 * t)} + c$$

where σ_t is the stress at time *t*. The *a+b+c* value referred as σ_0 that decayed over time *t* to plateau (*c*) or *asymptotic residual stress* with the decay rates K_1 and K_2.

Effect of heating on granular characteristics of the composites

The granular characteristics were determined as the *Water Absorption Index (WAI)* and *microstructure*. One mL of starch/flour suspension (0.67% w/v) in distilled water was shaken vigorously and allowed to stand for 10 min to absorb water at room temperature. The suspension was heated in a water-bath at 50, 60, 70, 80 and 90 °C for 30 min in a quiescent condition. The sample was cooled down to room temperature for 1 min and centrifuged at 14,000 rpm for 5 min (Spectrafuge 16M, USA). The supernatant liquid was discarded and the *WAI* was determined as the weight ratio of the water in the sediment and the dried weight of sample.

The *microstructure* of starch/flour granules in the presence of excess water before and after heating at 80 °C for 30 min and cooling was examined under the Lieca DME Light Microscope (USA). Ten fields of unheated starch/flour suspensions were observed for granule appearance and number. The sediment obtained after centrifugation was suspended in 0.5 mL distilled water and stained with 10% Lugol's iodine solution (Autio and Salmenkallio-Marttila, 2001). The microstructure of each starch/flour matrix in the presence of EA at the ratio of flour to protein as 2:1 was also observed. The EA suspension (0.33% protein w/v) was prepared by heating the EA suspension at 80 °C for 30 min and cooled down to room temperature as described above. The pre-heated EA suspension was then added to

starch/flour, shaken vigorously and the suspension was re-heated at 80 °C for 30 min prior to the examination under the light microscope.

RESULTS AND DISCUSSION

Thermal properties of RF and CS at different concentration were summarized in Table 1. Gelatinization temperature of the RF was higher than that of the CS (p<0.05), while the enthalpy of gelatinization (ΔH) and the gelatinization temperature range between T_{onset} and T_{end} of the CS was higher and wider than those of RF, respectively. This was likely to be due to the heterogeneity of granular size of the CS compared to the RF as illustrated in Figure 1. The flour/starch concentration effect on gelatinization temperature and enthalpy of both RF and CS was not evident due to the excess of water to complete gelatinization.

Due to the fact that the EA-flour composite gel was made of starch/flour and EA fractions, the stress-transmitting mechanisms could involve both entropic-driven mechanism caused by the entanglement of the microstructural element and the enthalpic contribution of the cross-links within the composite structure. Two-way ANOVA revealed that the type of flour filler had significant effect (p<0.05) on factor a and c, as well as the stress decay rate K_1 and K_2 (Figure 2). However, the calcium lactate concentration had significant effect on factors a, b and c (p<0.05) but not stress decay rate K_1 and K_2 (p≥0.05). The

magnitude of stress relaxed within the composite structure during prolonged compression was summarized in Figure 3, which showed that the EA-CS composite gel relaxed to the highest extent compared with others.

It is likely that the major microstructural element responsible for stress-carrying mechanisms in the two-stage heated protein-flour composite network is the gelatinized flour fraction due to the high content of flour filler compared to protein fraction. The more solid-like behavior of the EA-RF composite gel, compared with the EA-CS composite gel, was probably due to the ability of the RF filler to retain elasticity of the solid
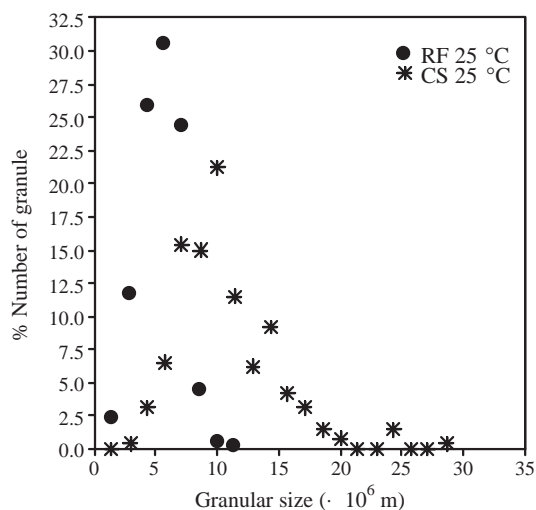

Figure 1 Particle size distribution of rice flour (RF) and cassava starch (CS) determined under the light microscope (N_{RF}=1298; N_{CS}=111).

Table 1 Thermal properties of food grade rice flour (RF) and cassava starch (CS) determined by DSC at the heating rate of 12.5 °C/min.

Flour type	Concentration (% w/w)	T_{onset} (°C)	T_{peak} (°C)	T_{end} (°C)	ΔH (J/g)
Rice flour	15	73.53[a]±0.19	78.01[a]±0.00	82.53[a]±0.05	9.803[b]±0.100
	30	72.09[b]±0.47	77.80[a]±0.30	83.21[a]±0.69	10.852[b]±0.018
Cassava starch	15	62.54[c]±0.19	67.28[c]±0.30	76.64[c]±0.28	14.037[a]±0.006
	30	62.23[c]±0.20	68.54[b]±0.00	79.17[b]±0.84	14.461[a]±0.443

Means in the same column followed by different superscript are significantly different (p<0.05).
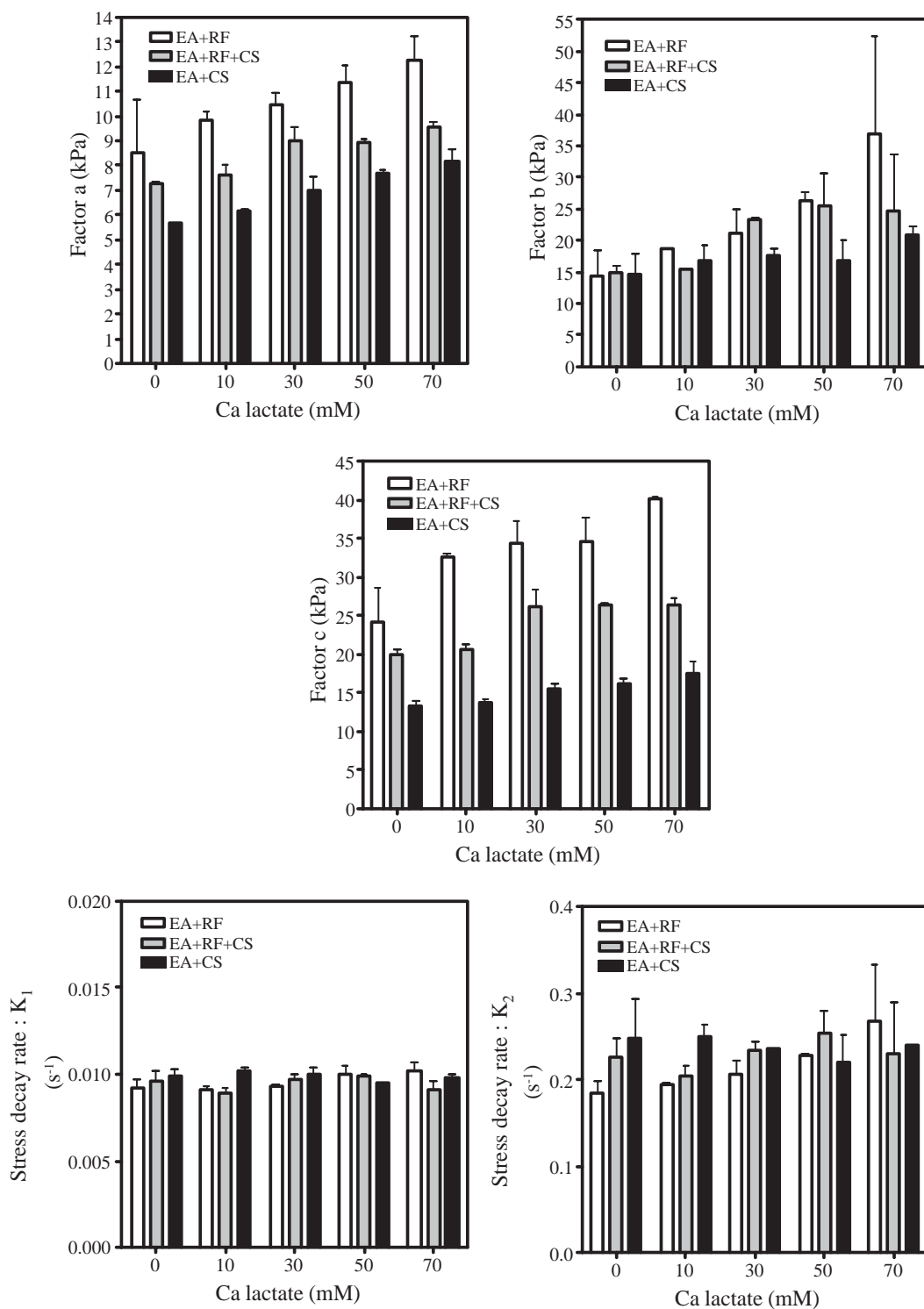
Figure 2 Two-phase exponential decay parameters describing stress relaxation characteristics of calcium-induced EA-flour composite gel after re-heating at 80°C for 30 min. Bars represent standard deviation.
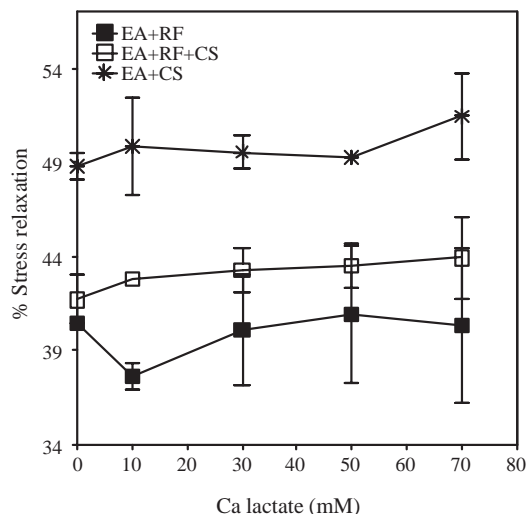
Figure 3 Effect of calcium lactate concentration on the stress relaxed after the EA-flour composite gel compressed for 300 s at 20% deformation. Bars represent standard deviation.

structure in the starch granules (Nussinovitch *et al.*, 1990). It was note that the gelatinization temperature of the RF was higher than that of the CS as shown in Table 1. In addition, the smaller granules of RF also resulted in the higher number of the RF granular particles per unit volume of the composite. The denser arrangement of the RF within the composite network may also be responsible for more solid-like behavior of the EA-RF, compared with the EA-CS.

Heating starch suspensions resulted in the changes in the microstructural geometry of the starch granules. The loss of original granular structure occurs after the water molecules penetrate into the granules. Thus, the resulting granules were mechanically weaker and more porous than the raw ones (Rao and Tattiyakul, 1999). We further explored the granular characteristics of RF and CS in distilled water after heating for 30 min at different temperature. Figure 4a illustrates that the heated RF absorbed water less than the CS when the temperature was raised

from 50 to 90 °C in quiescent condition and the mixture of RF and CS absorbed more water in linear fashion when the ratio of CS increased. However, the *WAI* profile of each composite as a function of temperature appeared to be different as shown in Figure 4b.

The *WAI* data were fitted by a nonlinear regression using the Grafit software package (Leatherbarrows, 1992) to characterize the influence of heating temperature on the *WAI* of RF and CS mathematically. The exponential equation (Eq. 1) could be used to describe the influence of temperature on the *WAI* of RF; while the power series model (Eq. 2) was best fit to the *WAI* of the CS (Table 2). The *WAI* response to heating temperature of the RF-CS composite seems to be a mix phenomenon of the two models.

$$y = a*exp^{(b*x)} \qquad \text{Eq.1}$$
$$y = a*x^b + c*x^d \qquad \text{Eq.2}$$

This study further showed that in addition to the pasting profile of RF and CS as previously reported (Hongsprabhas and Dit-udom-po, 2006), the mechanisms of starch/flour filler in reinforcing the mechanical strength of the EA-flour composite matrix also depended on volume fraction and strength of the hydrated granules. Figure 5 illustrates the microstructure of RF and CS after being heated at 80 °C for 30 min in the absence and presence of pre-heated EA and in the excess of water. It appears that the RF could, in part, reinforce the EA-RF composite gel via the aggregation of deformed (swollen, collapsed and disintegrated) amylose-rich fraction. It is likely that the presence of pre-heated EA could induce the aggregation of the RF through protein-protein interactions at the flour-protein interfaces. It should be noted that the commercial RF contained substantial amount of protein while the protein in commercial CS was not detected. Nevertheless, the nature of the RF and EA proteins interfacial interactions in water-in-water dispersions needs further investigation.

CONCLUSION

The structural reinforcement of starch/flour in the protein-starch composite matrix could be evident when the dispersed phase, or fillers, remained separated phase in the supporting protein matrix. The EA-CS composite gel was weaker and more compliant to external force than the EA-RF gel due to the differences in granular characteristics; e.g., large granular size, low number of particles per unit volume and the weakness of the hydrated granular structure after heating of the CS. The aggregation of the hydrated RF particles could further enhance the strength of protein-starch composite geometry during second heating step and the mechanical properties and applications of the composite structure accordingly.

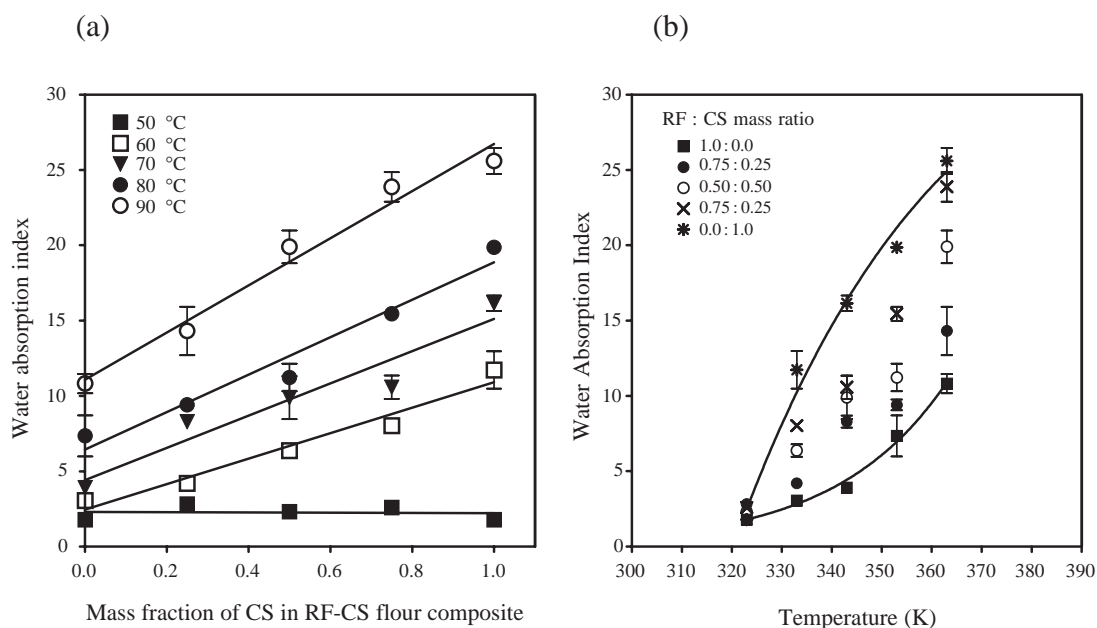(a)   (b)

Figure 4 Effect of cassava starch (CS) ratio in RF-CS flour composite on the water absorption index (*WAI*). Bars represent standard deviation.

Table 2 Non-linear regression parameters of the effect of heating temperature (*x*) on the *Water Absorption Index* (*y*) of rice flour (RF) and cassava starch (CS).

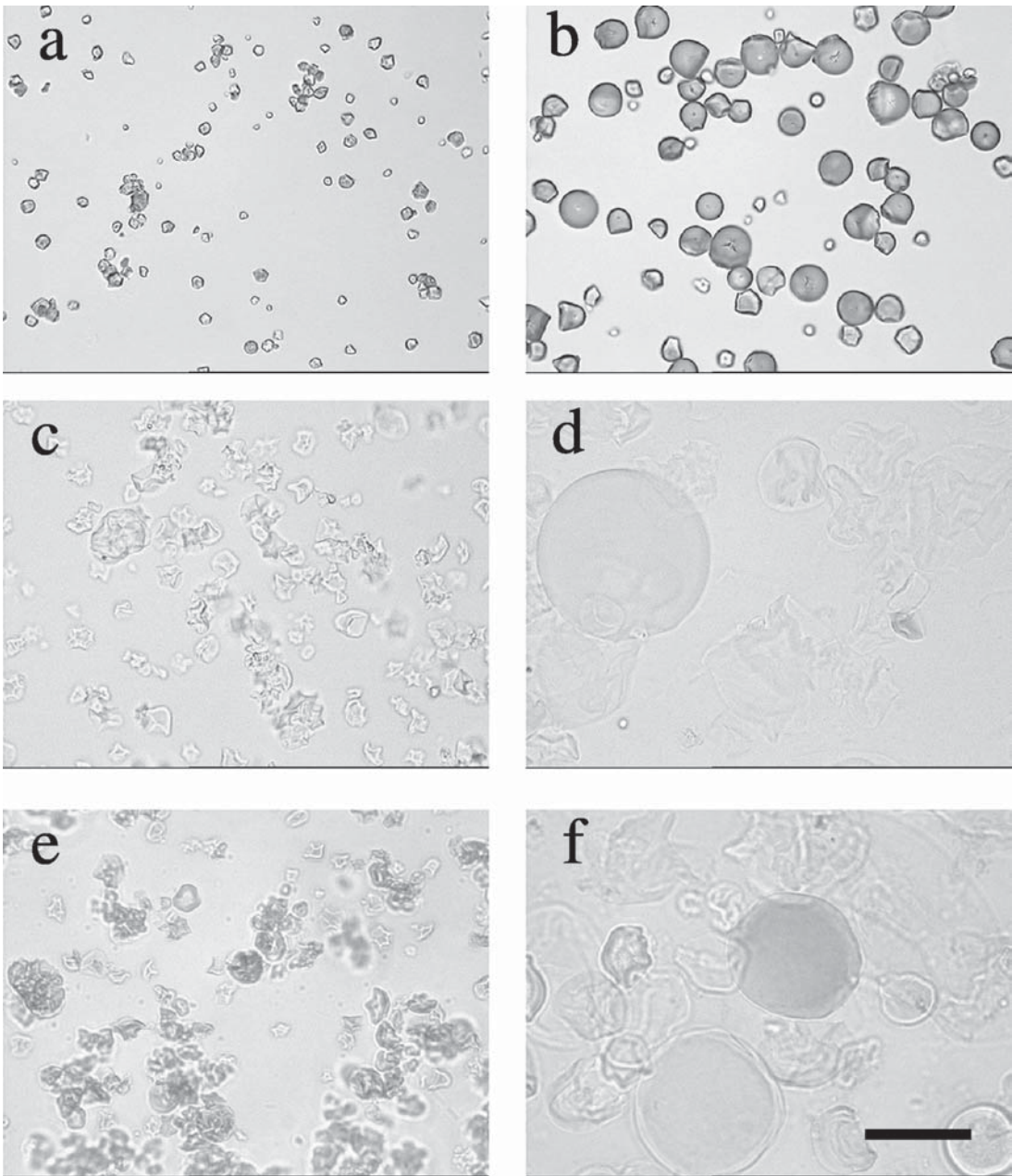| Flour type	Factor				
	a	*b*	*c*	*d*	*R²*
RF	8.71×10^{-7}	0.045	-	-	0.9622
CS	-6.71×10^{12}	-4.26	4.17×10^7	-2.181	0.9795

Figure 5 Light micrographs of food grade (a) rice flour and (b) cassava starch in distilled water before heating; (c) rice flour and (d) cassava starch after heating at 80 °C for 30 min in distilled water; and (e) rice flour and (f) cassava starch after heating at 80 °C for 30 min in pre-heated egg albumen suspension. Bar = 50 μm.

LITERATURE CITED

Aguilera, J.M. and P. Baffico. 1997. Structure-mechanical properties of heat-induced protein/cassava starch gels. **J. Food Sci.** 62(5) : 1048.

Aguilera, J.M. and E. Rojas. 1996. Rheological, thermal and microstructural properties of whey protein-cassava starch gels. **J. Food Sci.** 61(5): 962.

Aguilera, J.M. and G.V. Rojas. 1997. Determination of kinetics of gelation of whey protein and cassava starch by oscillatory rheometry. **Food Res. Intern**. 30(5): 349.

Association of Official Analytical Chemistry. 2000. **Official Methods of Analysis**. 17th ed. Arlington VA.

Autio, K. and M. Salmenkallio-Marttila. 2001. Light microscopic investigations of cereal grains, doughs and breads. **Lebensm.-Wiss.u.-Technol.** 34: 18.

Barbut, S. and E.A. Foegeding. 1993. Ca^{2+}-induced gelation of pre-heated whey protein isolate gels. **J. Food Sci.** 58: 867.

Doi, E. 1993. Gels and gelling of globular proteins. **Trends Food Sci. Technol**. 4: 1.

Hongsprabhas, P. and S. Dit-udom-po. 2006. Influence of flour type on mechanical properties of calcium-induced egg albumen-flour composite gel. www.bepress.com/ijfe/vol2/iss1/art2.

Leatherborrow, R.J. 1992. **Grafit version 3.0**. Erithacus Software Ltd., Staines.

McClements, D.J., F.J. Monahan and J. E. Kinsella. 1993. Effect of emulsion droplets on the rheology of whey protein isolate gels. **J. Texture Stud.** 24: 411.

Nussinovitch, A., M.M. Ak, M.D. Normand and M.M. Peleg. 1990. Characterization of gellan gels by uniaxial compression, stress relaxation and creep. **J. Texture Stud.** 21: 37.

Rao, M.A. and J. Tattiyakul. 1999. Granule size and rheological behavior of heated tapioca starch dispersions. **Carbohydrate Polymers.** 38: 123.

Richardson, R.K., G. Robinson, S.B. Ross-Murphy and S. Todd. 1981. Mechanical spectroscopy of filled gelatin gels. **Polymer Bull.** 4: 541.

Ross-Murphy, S.B. and S. Todd. 1983. Ultimate tensile measurements of filled gelatin gels. **Polymer** 24: 481.

Wongsasulak, S., T. Yoovidhya, S. Bhumiratana and P. Hongsprabhas. 2004. Effect of aging temperature on diffusion and microstructure of egg albumen-cassava starch composite film. **Presented at the IFT 2004 Annual Meeting and Food Expo**, Las Vegas.

Wongsasulak, S., T. Yoovidhya, S. Bhumiratana, P. Hongsprabhas, D.J. McClements and J. Weiss. 2006. Thermo-mechanical properties of egg albumen - cassava starch composite films containing sunflower-oil droplets as a function of moisture content. **Food Research Intern**. 39(3): 277.

Tang, J., J. Lelievre, M.A. Tung and Y. Zeng. 1994. Polymer and ion concentration effects on gellan gel strength and strain. **J. Food Sci.** 59: 216.