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Chitosan effects on physical properties, texture, and microstructure of flat rice noodles

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ABSTRACT

The use of chitosan as a natural preservative has increased for rice-based products, but little is known about chitosan effects on product quality. This work elucidated chitosan effects on the physical properties (moisture content, pH, and color), texture, and microstructure of flat rice noodles. Chitosan solutions (0.33 and 0.50 g chitosan/100 mL acetic acid) were added to rice flour noodles. Chitosan exhibited no effect on moisture contents, but it decreased the rice noodle pH and whiteness. Confocal laser scanning micrographs revealed that acetic acid caused protein network formation in rice noodles. Chitosan did not affect textural properties of rice flour gel because the acetic-acid-induced protein networks inhibited chitosan effects. During storage at 30 °C for 5 d, chitosan retarded increases in the hardness and gumminess and decreases in the cohesiveness and springiness of rice flour gel. Results show that chitosan is useful as a preservative in rice flour noodles without adversely affecting noodle quality.

1. Introduction

Flat rice noodles, made from high amylose content rice flour, are extremely popular noodles in Southeast Asia (Fu, 2008). However, fresh flat rice noodles spoil easily in a single day because of microorganism activity, so food preservatives, especially benzoic acid and sodium benzoate, are used widely to extend the shelf-life of rice noodles. Today demand for non-artificial food preservatives has increased, so natural preservatives are preferred. Chitosan, derived from chitin, has high potential as a natural preservative for rice noodles because it exhibits wide-spectrum antimicrobial activity against bacteria, yeast, and fungi (Juneja, Dwivedi, & Yan, 2012; No, Meyers, Prinyawiwatkul, & Xu, 2007). Chitosan inhibits spoilage microorganisms in cooked rice and rice cake with proven efficacy (Lee, Lee, & Rhim, 2000; Rachtanapun, Tantala, Klinmalai, & Ratanasumawong, 2015; Tsai, Tsai, Lee, & Zhong, 2006). Moreover, chitosan is an approved food additive in many countries including Korea, Japan, and the USA (Hayes, 2012; No & Meyers, 2004; Rachtanapun et al., 2015). Chitosan is applied in food by coating the food surface or by addition directly into food. Effects of chitosan coating on food quality have been well studied, but few reports describe direct effects of chitosan addition on food quality, especially for rice-based products. The effects of added chitosan on food quality depend on the target food type. No and Meyers (2004) reported that chitosan increases the brightness and yellowness of tofu (soy bean curd). In contrast, chitosan reportedly has no effect on the whiteness of cooked rice (Rachtanapun et al., 2015).

Food texture determines the consumer acceptance of noodles. Hardness of sweet potato starch noodles is increased by the addition of chitosan (Baek, Cha, & Lim, 2001), although chitosan shows no effects on the sensory attributes of amaranth pasta (Del Nobile, Benedetto, Suriano, Conte, Corbo, & Sinigaglia, 2009). Lee et al. (2000) reported no difference in sensory evaluation between the texture of wet noodles with chitosan and control noodles. Although numerous studies have examined chitosan effects on noodle texture, no clear explanation has been offered for how chitosan affects the texture of those noodles, especially from a microstructural viewpoint. Because the effects of chitosan on noodle texture depend strongly on the noodle type, chitosan effects on the target noodle texture must be elucidated before using chitosan as an additive. No report of the relevant literature describes a study of chitosan effects on the physical properties, texture, and microstructure of flat rice noodles.

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This work was undertaken to investigate the effects of chitosan on the physical properties (color, pH, and moisture content) and texture of flat rice noodles. The effects of acetic acid and chitosan on the flat rice noodle microstructure were first observed using confocal laser scanning microscopy (CLSM). The relation between the rice gel texture and changes in the noodle microstructure attributable to the presence of acetic acid and chitosan was examined in this work. Furthermore, chitosan effects on the moisture content, pH, color, and texture of flat rice noodles during storage were examined. The results presented herein are expected to be helpful as guidance for the widened application of chitosan in rice-based products.

2. Materials and methods

2.1. Materials

Flake crab chitosan (polymer type, MW 280,000 Da, 94.78% degree of deacetylation; Ta Ming Enterprises Co., Ltd., Samutsakhon, Thailand) was used for this study. Glacial acetic acid was purchased from Merck and Co. Inc., Germany.

2.2. Chitosan solution preparation

Crab chitosan flakes of 3.3 g and 5 g were dissolved in 1000 mL of 0.33 mL acetic acid/100 mL deionized water to achieve the final respective concentrations of 0.33 g and 0.50 g crab chitosan per 100 mL acetic acid. Crab chitosan solutions were prepared, sterilized, and stored according to the method described by Rachtanapun et al. (2015).

2.3. Preparation of rice flour and rice starch

Rice grains (Leung 11 cultivar) were purchased from a rice mill in Kalasin, Thailand. The rice grains were wet milled according to Sangpring, Fukuoka, and Ratanasumawong (2015). The rice flour consisted of 10.69 g moisture/100 g dry sample, 6.02 g protein/100 g, 0.23 g ash/100 g, and 0.11 g fat/100 g. The amylose content of rice flour analyzed using the method described by Juliano (1971) was 36.51 g/100 g. This value agrees with the amylose contents of six Thai rice cultivars, which were 32–36 g/100 g (Yoenyongbuddhagal & Noomhorm, 2002).

Rice starch was prepared from rice flour by following a modified method of Lumdubwong and Seib (2000). To extract the protein from the rice flour, 0.05 mol/L NaOH solution was added to the rice flour in the ratio 5:2. After extraction, the rice starch was washed with deionized water, dried at 40 °C, milled, screened through a 0.150 mm sieve, packed in plastic bags, and stored at 10 °C until use. The rice starch consisted of 10.44 g moisture/100 g dry sample, 0.10 g protein/100 g, 0.17 g ash/100 g, and 0.01 g fat/100 g. The amylose content of the rice starch, as analyzed using the method described by Juliano (1971), was 38.80 g/100 g.

2.4. Rice noodle preparation

Fresh rice flour noodles were prepared according to the method described by Sangpring et al. (2015). Rice flour slurry was prepared by mixing 40 g of rice flour with 60 g of either deionized water, 0.33 mL acetic acid/100 mL deionized water, 0.33 g chitosan/100 mL acetic acid, or 0.50 g chitosan/100 mL acetic acid. Sixty grams of each slurry were poured evenly on a stainless tray and were steamed at 100 °C for 3 min. The rice noodle sheet was removed from the tray and cut into 20 cm × 1.5 cm strands.

Rice starch noodles with either deionized water, 0.33 mL acetic acid/100 mL deionized water, 0.33 g chitosan/100 mL acetic acid, or 0.50 g chitosan/100 mL acetic acid were prepared using the method used for the preparation of the rice flour noodles described above.

2.5. Moisture content and pH of rice noodles

The moisture contents of the rice starch or rice flour noodle samples were measured according to AOAC method 935.29 (AOAC, 2000). The pH of each rice noodle sample was measured according to AOAC method 943.02 (AOAC, 2000).

2.6. Color of rice noodles

After steaming, the rice flour noodle sheets were cut into 5 cm × 5 cm sheets. The color of each type of rice noodle sheet was measured using a colorimeter (Miniscan XE; Hunter Associates Laboratory Inc., USA) based on the CIE system with color values of L^*, a^* and b^*. The whiteness index (WI) of each sample was calculated using Eq. (1) (Rachtanapun et al., 2015).

$$WI = 100 - \left[(100 - L^*)^2 + a^{*2} + b^{*2}\right]^{1/2} \quad (1)$$

The whiteness of each type of rice noodle was averaged from at least 10 noodle sheets. All measurements were conducted in triplicate.

2.7. Texture profile analysis of rice gels

Texture profile analysis (TPA) method is usually applied to measure the noodle texture using a texture analyzer because this method collects large amounts of information related to the noodle texture at one time. To acquire accurate data, a machine load cell should match well with the magnitude of acquired force from sample. Cham and Suwannaporn (2010) reported that fresh flat noodle hardness was only 0.13 kg. Therefore, to increase the force to match with the load cell, the sample thickness was increased and prepared in the form of rice gels according to the method described by Huang, Kennedy, Li, Xu, and Xie (2007), with some modifications. The ratio between rice flour or rice starch to solutions was equal to the ratio used in preparing noodles. Rice flour or rice starch slurry was prepared by mixing 40 g of rice flour or rice starch with 60 g of either deionized water, 0.33 mL acetic acid/100 mL deionized water, 0.33 g chitosan/100 mL acetic acid, or 0.50 g chitosan/100 mL acetic acid. Sixty grams of either slurry of rice flour or rice starch was poured evenly in a 7.3 cm × 5.4 cm × 1 cm deep aluminum tray, after which it was steamed for 15 min. Rice gel of both types was cut into cubes 1 cm × 1 cm × 1 cm. Texture profile analysis (TPA) of both rice gels was conducted using a texture analyzer (TA-XT PLUS; Stable Micro Systems, Ltd., Surrey, UK) with a 50 kg load cell according to the modified method of Baek et al. (2001) and Huang et al. (2007). Compression was repeated at 1.0 mm/s with a flat-faced 100-mm-diameter cylinder probe at 50% strain. The pause between the first and second compressions was 60 s. Hardness, cohesiveness, springiness, and gumminess were analyzed from the measured profile. At least 10 samples were measured for each treatment. Each measurement was taken in triplicate.

2.8. Confocal laser scanning microscopy (CLSM)

The respective microstructural features of the rice flour and rice starch noodles prepared with deionized water, 0.33 mL acetic acid/100 mL deionized water, or 0.50 g chitosan/100 mL acetic acid were observed using a confocal laser scanning microscope (LSM5 PASCAL; Carl Zeiss Inc., Göttingen, Germany). Sample preparation was conducted according to Sangpring et al. (2015). Rice flour or rice starch noodle sheets were cut into 5 mm × 5 mm pieces. A solution of fluorescein isothiocyanate (FITC; 0.01 g/100 mL 95% ethanol) (Sigma-Aldrich Corp., St. Louis, USA) was added to the sample to observe the starch location. A solution of rhodamine B (0.01 g/100 mL 95% ethanol) (Invitrogen Corp., Carlsbad, USA) was added to the sample to observe the protein location (Tromp, Nicolas, Van de velde, & Paques, 2003). Each sample was observed under a confocal laser scanning microscope. A He-Ne laser was used with respective excitation wavelengths for FITC and rhodamine B of 488 and 543 nm.

2.9. Fluorescence microscopy

Chitosan in rice starch noodles was observed using fluorescence microscopy. The rice starch noodle sections were prepared with various solutions according to a modified method of Thammathongchat, Fukuoka, and Watanabe (2005). Each rice noodle strand was cut into 1 cm × 1 cm pieces. Then, the rice noodle samples were embedded in frozen section media (FSC 22) and were immersed in liquid nitrogen. The frozen sample blocks were kept in the freezer at −80 °C until sectioning. Samples were cut into 10-μm-thick sections using a cryostat (CM 1850; Leica Microsystems) at −20 °C.

Each rice noodle section was stained with rhodamine B isothiocyanate (0.01 g/100 mL 95% ethanol) (Sigma-Aldrich Japan K.K., Tokyo, Japan). Samples were observed under a fluorescence microscope (BZ-9000; Keyence Co., Osaka, Japan) using 540 ± 12.5 nm excitation wavelength and 605 ± 27.5 nm absorption wavelength.

2.10. Effects of chitosan on moisture content, pH, color, and textural properties of rice flour noodles during storage

Rice flour noodles or rice flour gel samples were packed in plastic bags (PP) and were kept in an incubator at 30 °C. Moisture content, pH, and whiteness of the rice flour noodles, and rice flour gel texture were measured. All measurements were taken in triplicate.

2.11. Statistical analysis

A completely randomized design (CRD) was used to analyze the data related to the physical properties of the rice flour noodles and the texture data of the rice starch and rice flour gels. Mean values were compared using analysis of variance (ANOVA). Significant differences among mean values were inferred using Duncan's multiple range test for comparing treatments ($p \leq 0.05$) in a software package (SPSS ver. 12.0; SPSS Inc., Chicago, IL, USA).

A split-plot design was used to analyze the physical properties of the rice flour noodles during storage at 30 °C: the main factor was treatment; the sub factor was days. The mean data of each combination of treatments were analyzed using ANOVA and Duncan's multiple range test for comparing treatments ($p \leq 0.05$). All calculations were done using software (SPSS ver. 12.0; SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Effects of chitosan on moisture content, pH, and whiteness of flat rice noodles

The moisture content, pH, and whiteness of rice flour noodles are shown in Table 1. The addition of acetic acid and chitosan had no effect on the moisture content of the rice flour noodles compared with the control (noodles in deionized water). The same result was observed by Rachtanapun et al. (2015) who reported that the addition of crab chitosan had no effect on the moisture content of cooked rice. As expected, the pH value of rice flour noodles with the chitosan was lower than that with deionized water because of the presence of acetic acid.

The whiteness of the rice flour noodles with 0.33 g chitosan/100 mL acetic acid was similar to the control, but the whiteness of noodles with 0.5 g chitosan/100 mL acetic acid was lower than in other samples. The chitosan flakes were yellow to brown because the chitin was treated with alkali in the N-deacetylation process during chitosan preparation (Yen, Yang, & Mau, 2009). Moreover, the chitosan solutions changed from yellow to darker yellow after sterilization because of the Maillard reaction (Yang, Zhao, Liu, Ding, & Gu, 2007). Therefore, an increased chitosan concentration decreased the whiteness of the noodles because of the color of chitosan.

3.2. Effect of chitosan on the rice flour gel texture

When chitosan is applied to foods, it must be dissolved in acid solution, normally acetic acid. However, the presence of acid might alter the food quality. Acetic acid decreases the hardness and increased the stickiness of cooked rice because the acetic acid promotes water adsorption of amylopectin in rice starch granule and dissolves rice proteins from rice starch granule surface (Ohishi, Kasai, Shimada, & Hatae, 2003, 2007). Moreover, the presence of chitosan might alter the texture of flat rice noodles. Huang et al. (2007) reported that the texture of rice starch gel is dependent on the type of added non-starch polysaccharides. Charoenrein, Tatirat, Rengsutthi, and Thonghgam (2011) reported that the hardness of unfrozen rice starch gel with konjac glucomannan was lower than without konjac glucomannan. Therefore, we expected that the presence of acetic acid and chitosan might provide a softer rice noodle texture. Against our expectations, no difference was found between the textural properties of rice flour gels with chitosan and the control (Table 2). However, this result agrees with those of the addition of chitosan in amaranth pasta conducted by Del Nobile et al. (2009). They reported that amaranth pasta with chitosan (2 and 4 g/kg) exhibits no difference in sensorial tests. Therefore, we infer that chitosan has no effect on the rice flour noodle texture.

3.3. Microstructure of rice flour noodles with chitosan

Confocal laser scanning micrographs revealed the distribution of starch and protein in the rice flour noodles (Fig. 1). Starch is

Table 1
Moisture content, pH, and whiteness of rice flour noodles with various chitosan concentrations.

Parameter	Deionized water	0.33 mL Acetic acid/100 mL deionized water	0.33 g Chitosan/100 mL acetic acid	0.50 g Chitosan/100 mL acetic acid
Moisture content (%)	58.25 ± 1.37^{a}	59.17 ± 1.59^{a}	58.46 ± 2.04^{a}	58.49 ± 1.30^{a}
pH	6.16 ± 0.13^{b}	4.38 ± 0.14^{a}	4.34 ± 0.06^{a}	4.54 ± 0.12^{a}
Whiteness	86.18 ± 0.38^{b}	87.47 ± 0.91^{c}	85.68 ± 0.11^{b}	83.81 ± 0.85^{a}

Values are mean ± S.D. of three independent determinations.
^{a,b,c} Means in the same row followed by different letters are significantly different ($p \leq 0.05$).

Table 2
Textural characteristics of rice flour and rice starch gels with various chitosan concentrations.

	Treatment	Hardness (N)	Springiness	Cohesiveness	Gumminess (N)
Rice flour	Deionized water	10.21 ± 0.43[a]	0.73 ± 0.04[a]	0.90 ± 0.01[a]	9.20 ± 0.45[a]
	0.33 mL Acetic acid/100 mL deionized water	10.70 ± 0.96[a]	0.71 ± 0.05[a]	0.89 ± 0.03[a]	9.46 ± 0.63[a]
	0.33 g Chitosan/100 mL acetic acid	10.74 ± 0.38[a]	0.75 ± 0.03[a]	0.90 ± 0.01[a]	9.78 ± 0.25[a]
	0.50 g Chitosan/100 mL acetic acid	10.68 ± 0.18[a]	0.74 ± 0.03[a]	0.90 ± 0.01[a]	9.62 ± 0.17[a]
Rice starch	Deionized water	10.43 ± 0.21[b]	0.70 ± 0.03[a]	0.91 ± 0.01[a]	9.42 ± 0.17[b]
	0.33 mL Acetic acid/100 mL deionized water	11.33 ± 0.24[c]	0.73 ± 0.06[a]	0.89 ± 0.01[a]	10.04 ± 0.08[b]
	0.33 g Chitosan/100 mL acetic acid	7.51 ± 0.14[a]	0.68 ± 0.04[a]	0.94 ± 0.00[b]	7.05 ± 0.12[a]
	0.50 g Chitosan/100 mL acetic acid	7.72 ± 0.60[a]	0.72 ± 0.03[a]	0.94 ± 0.01[b]	7.23 ± 0.51[a]

Values are mean ± S.D. of three independent determinations.
[a,b,c] Means in the same column followed by different letters are significantly different ($p \leq 0.05$).

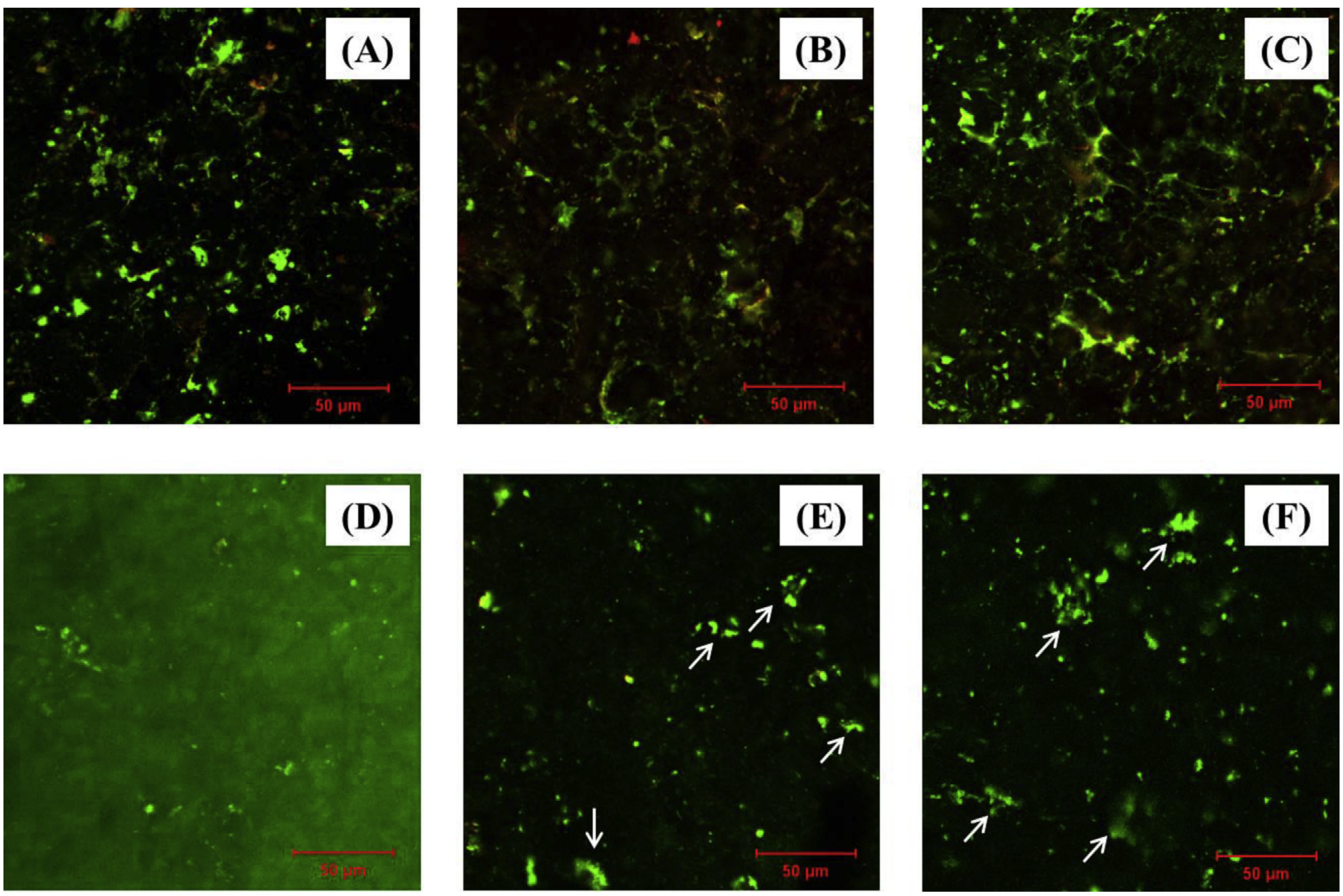

Fig. 1. Confocal laser scanning micrographs of rice starch and rice flour noodles with various solutions: (A) rice flour noodles with deionized water; (B) rice flour noodles with acetic acid; (C) rice flour noodles with 0.50 g chitosan/100 mL acetic acid; (D) rice starch noodles with deionized water; (E) rice starch noodles with acetic acid; (F) rice starch noodles with 0.50 g chitosan/100 mL acetic acid. Arrows in (1E) and (1F) respectively indicate groups of starch and groups of starch and chitosan.

shown mainly as green because of the starch-FITC interaction, whereas protein is shown mainly as red because of the protein–rhodamine B interaction. The main structure of rice flour noodles with deionized water (Fig. 1A) is a starch network, as shown in the distribution of green color groups through the noodle. Moreover, there were protein groups (red color) as well as the starch groups. This structure resembled the structures of rice flour noodles reported by Kerdsrilek and Garnjanagoonchorn (2014) and Sangpring et al. (2015).

In contrast, clear development of a starch and protein network in the rice flour noodles with acetic acid (Fig. 1B) was observed. This might have occurred because the pH of these rice noodles with acetic acid was lower than the isoelectric point (pI) of rice protein. The pI of oryzanin protein, the main protein in rice, is 6.5–7.5 (Wen & Luthe, 1985). The pH of rice flour noodles with acetic acid was 4.38, which was lower than the pI, so rice protein might slightly unfold because of electrostatic repulsion within a molecule and consequently form a network. Similar to the structure of rice flour noodles with acetic acid, the clear starch–protein network existed in rice flour noodles with chitosan (Fig. 1C) because of the presence of acetic acid. However, a more developed network was observed as being more fibrous and with mixed color areas. Because chitosan can be stained with both rhodamine B as a red color and with FITC as a green color (Klinkesorn & Namatsila, 2009; Tromp et al., 2003), the increase in the mixed color area might be attributed to the presence of chitosan in that area. This result suggests that chitosan is embedded in the starch–protein network.

Formation of the protein network of the rice flour noodles attributable to acetic acid might be the key cause of retarded textural change of rice flour gels with chitosan. To prove this hypothesis, the effects of acetic acid and chitosan on the rice starch noodle texture and microstructure were investigated.

3.4. Textural properties of rice starch gels and rice starch noodle microstructure

Although chitosan has no effect on the rice flour noodle texture, it elicited significant changes in the rice starch gel texture (Table 2). The hardness and gumminess of rice starch gels with chitosan added were the lowest. Furthermore, the cohesiveness of rice starch gels with chitosan was the highest among samples. These facts suggest that the texture of rice starch noodles with chitosan is soft and cohesive. Similar to the result of Charoenrein et al. (2011), the rice starch gel hardness was lower with konjac glucomannan than without. Apparently, konjac glucomannan retarded the swollen starch granule aggregation. Actually, chitosan might have interrupted the aggregation of starch molecules in the rice starch gel in our work as well.

To elucidate the cause of soft texture of rice starch gel with chitosan, the rice starch noodle microstructure was observed under CLSM. All types of rice starch noodle samples were displayed as green (Fig. 1D, E, and F) because of their extremely low amounts of protein remaining in the rice starch (0.1 g/100 g). The smooth, continuous green color area in the rice starch noodles revealed the main structure of these noodles as a continuous starch network. In contrast, the addition of acetic acid to the rice starch noodles caused the formation of several clusters of starch (Fig. 1E). Rice starch noodles with chitosan (Fig. 1F) showed green clusters resembling those of the rice starch noodles with the presence of acetic acid but the green clusters in the samples with chitosan were slightly larger than those found in the rice starch noodles with acetic acid. Because FITC can stain chitosan by covalent labeling between isothiocyanate derivatives and chitosan (Velde, Weinbreck, & Edelman, 2003), the larger green clusters of the samples with chitosan might have been clusters of chitosan.

To clarify whether the green clusters in the rice starch noodles were chitosan, or not, rhodamine B isothiocyanate was used to stain the rice starch noodles with chitosan, which can link to the isothiocyanate derivatives by a covalent bond (Ma et al., 2008). Consequently, the chitosan became visible under a fluorescence microscope. Fluorescence micrographs of the rice starch noodles with deionized water (Fig. 2A) and acetic acid (Fig. 2B) showed tiny red clusters attributable to the low amounts of protein remaining in the rice starch (0.1 g/100 g). Fluorescence micrographs of rice starch noodles with chitosan (Fig. 2C) revealed some red clusters that were larger than those found in the samples without chitosan (Fig. 2A and B). Because of the low amounts of protein remaining in the rice starch, the larger red clusters in the rice starch noodles with chitosan (Fig. 2C) should be regarded as indicating chitosan. The rice starch gel strength is attributable to the starch network (amylose network). However, the presence of chitosan might interrupt the formation of starch networks (Figs. 1F and 2C), thereby weakening their structure. The rice starch gel hardness decreased; the cohesiveness increased (Table 2).

Differences of textures of rice flour and rice starch gels with chitosan confirm the protein network formation in the rice flour noodles because of the presence of acetic acid is the key factor regulating the rice flour noodle texture with chitosan added. The existence of a protein network in rice flour gel might restrict the interruption by chitosan and maintain the noodle structure. According to our findings, it might be useful to explain the change in texture of other starch or flour noodles because of the presence of chitosan. In wheat-based noodles, protein networks (gluten networks) might retard the acid and chitosan effects on noodle texture, which might explain the lack of chitosan effects on wet noodle texture (wheat flour based noodles) reported by Lee et al. (2000). In contrast, increased hardness of sweet potato starch noodles with added chitosan (Baek et al., 2001) might be attributed to the lack of protein networks.

3.5. Effects of chitosan on moisture content, pH, whiteness, and texture of rice flour noodles during storage at 30 °C

Spots of microorganisms were found on rice flour noodle surfaces with deionized water after 1 d of storage and on rice flour noodle surfaces with acetic acid and on noodles with chitosan after 5 d. Only the samples of 0 d and 1 d of rice flour noodles with deionized water and samples up to 5 d for the rice flour noodles with acetic acid and the noodles with chitosan were measured. Table 3 shows the moisture content, pH, and whiteness of rice flour noodles having various chitosan concentrations during storage at 30 °C over 5 d. No difference was found in the moisture contents of all samples. It remained constant during storage. The pH of the noodles with deionized water increased slightly during storage, although the pH of samples with acetic acid and chitosan remained constant during storage. Noodles with acetic acid had the lowest pH during storage, followed by the rice noodles with chitosan and deionized water.

The whiteness of the rice flour noodles with deionized water increased during storage for 1 d because starch retrogradation made the noodles more opaque. In contrast, the whiteness of the rice flour noodles with acetic acid and chitosan remained constant

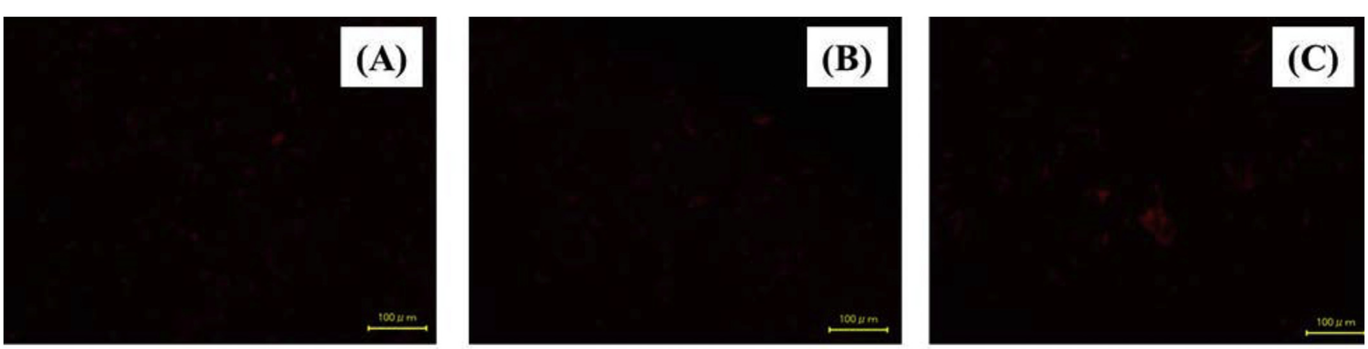

Fig. 2. Fluorescence micrographs of rice starch noodles with various added solutions: (A) rice starch noodles with deionized water; (B) rice starch noodles with acetic acid; (C) rice starch noodles with 0.50 g chitosan/100 mL acetic acid.

Table 3
Moisture content, pH and whiteness of rice flour noodles with various chitosan concentrations during storage at 30 °C for 5 d.

Parameter	Day	Treatment			
		Deionized water	0.33 mL Acetic acid/100 mL deionized water	0.33 g Chitosan/100 mL acetic acid	0.50 g Chitosan/100 mL acetic acid
Moisture content (%)	0	60.79 ± 1.30^{bc}	59.90 ± 1.78^{abc}	60.97 ± 0.45^{c}	60.83 ± 0.40^{bc}
	1	59.32 ± 0.23^{ab}	59.39 ± 1.13^{ab}	60.37 ± 0.38^{abc}	60.49 ± 0.56^{abc}
	3		59.11 ± 0.78^{a}	60.23 ± 0.17^{abc}	60.08 ± 0.35^{abc}
	5		59.12 ± 0.85^{a}	59.81 ± 0.35^{abc}	60.03 ± 0.39^{abc}
pH	0	6.12 ± 0.06^{f}	4.24 ± 0.02^{a}	4.45 ± 0.04^{bcd}	4.58 ± 0.01^{de}
	1	6.47 ± 0.11^{g}	4.23 ± 0.01^{a}	4.42 ± 0.01^{bc}	4.62 ± 0.00^{e}
	3		4.30 ± 0.06^{ab}	4.47 ± 0.04^{cde}	4.59 ± 0.07^{de}
	5		4.23 ± 0.08^{a}	4.47 ± 0.08^{cde}	4.61 ± 0.13^{e}
Whiteness	0	87.57 ± 0.10^{f}	88.39 ± 0.17^{gh}	86.78 ± 0.09^{de}	84.81 ± 0.03^{b}
	1	88.19 ± 0.15^{g}	88.87 ± 0.09^{h}	87.19 ± 0.21^{ef}	85.63 ± 0.03^{c}
	3		88.16 ± 1.03^{g}	86.94 ± 0.18^{de}	84.27 ± 0.23^{a}
	5		88.75 ± 0.09^{h}	86.53 ± 0.30^{d}	84.85 ± 0.11^{b}

Values are mean ± S.D. of three independent determinations.
[a,b,c,d,e,f,g,h] Means followed by different letters are significantly different ($p \leq 0.05$).

during storage. The rice flour noodles with chitosan exhibited the lowest whiteness among samples. Those results resemble results for rice cooked with chitosan, which showed no change in the moisture content, pH, or whiteness during storage at 37 °C (Rachtanapun et al., 2015).

The textural characteristics of rice flour gel during storage at 30 °C for 5 d are shown in Table 4. At day 1, the hardness and gumminess of gel with deionized water increased significantly, although the hardness and the gumminess of gel with acetic acid and gel with chitosan remained constant. The increased hardness possibly resulted from starch retrogradation (Satmalee & Charoenrein, 2009). This result suggests that acetic acid and chitosan retards the starch retrogradation of rice flour gel.

After 1 d, the hardness and gumminess of both the rice flour gel with acetic acid and the gel with chitosan increased, while the cohesiveness and springiness decreased. Because the moisture content levels of both rice flour gels during storage were not significantly different ($p > 0.05$) (Table 3), no change in textural properties occurred because of the loss of moisture, but rather it resulted from the change in the structure of the rice flour gel: starch retrogradation. Increased hardness and gumminess and decreases in the cohesiveness and springiness of the rice flour gel with chitosan were slower than those with acetic acid. Kerch et al. (2008) reported that chitosan can retard staling of bread (starch retrogradation) during storage. The presence of guar gum reduced the retrogradation of corn starch and amaranth starch because of interaction between the guar gum and amylose (Sudhakar, Singhal, & Kulkarni, 1996). Considering results of these earlier studies, the results of slower changes in the hardness, gumminess, cohesiveness, and springiness of the rice flour gel with chitosan might have been attributable to the lesser retrogradation of starch caused by the interruption of chitosan in the starch network. Therefore, it can be concluded that chitosan can retard the change in the texture of rice flour gel during storage at 30 °C, which suggests that the use of chitosan in rice flour noodles can maintain noodle quality during storage.

4. Conclusion

This report is the first of the relevant literature to investigate the effect of chitosan on the physical properties, texture, and microstructure of flat rice flour noodles. The chitosan addition decreased the noodle whiteness, although chitosan had no effect on the moisture content. Results showed that the formation of a protein network attributable to the presence of acetic acid in rice flour noodles was the key factor regulating the texture of rice noodles

Table 4
Textural characteristics of rice flour gels with various chitosan concentrations during storage at 30 °C for 5 d.

Parameter	Day	Treatment			
		Deionized water	0.33 mL Acetic acid/100 mL deionized water	0.33 g Chitosan/100 mL acetic acid	0.50 g Chitosan/100 mL acetic acid
Hardness (N)	0	10.07 ± 0.33^{a}	10.58 ± 0.21^{a}	10.27 ± 0.21^{a}	10.30 ± 0.17^{a}
	1	13.51 ± 0.60^{b}	10.93 ± 0.63^{a}	10.81 ± 0.27^{a}	10.30 ± 0.52^{a}
	3		20.71 ± 0.37^{d}	19.58 ± 1.15^{c}	20.58 ± 0.28^{d}
	5		21.67 ± 0.65^{e}	20.08 ± 0.32^{cd}	20.83 ± 0.30^{d}
Cohesiveness	0	0.89 ± 0.01^{de}	0.90 ± 0.01^{e}	0.89 ± 0.01^{de}	0.90 ± 0.01^{de}
	1	0.87 ± 0.01^{d}	0.88 ± 0.01^{de}	0.87 ± 0.01^{de}	0.88 ± 0.01^{de}
	3		0.77 ± 0.04^{c}	0.72 ± 0.02^{b}	0.70 ± 0.01^{ab}
	5		0.68 ± 0.02^{a}	0.73 ± 0.01^{b}	0.72 ± 0.04^{b}
Gumminess (N)	0	9.20 ± 0.12^{a}	9.07 ± 0.26^{a}	9.56 ± 0.11^{a}	8.97 ± 0.27^{a}
	1	11.67 ± 0.44^{b}	9.79 ± 0.29^{a}	9.43 ± 0.25^{a}	9.34 ± 0.13^{a}
	3		13.62 ± 1.29^{cd}	12.33 ± 0.60^{b}	14.66 ± 0.68^{d}
	5		15.00 ± 1.16^{d}	14.30 ± 0.40^{cd}	13.43 ± 0.62^{c}
Springiness	0	0.71 ± 0.02^{fgh}	0.74 ± 0.01^{hij}	0.75 ± 0.01^{ij}	0.76 ± 0.02^{j}
	1	0.66 ± 0.03^{cde}	0.67 ± 0.04^{de}	0.68 ± 0.02^{def}	0.69 ± 0.03^{efg}
	3		0.65 ± 0.01^{cd}	0.67 ± 0.02^{de}	0.72 ± 0.03^{ghi}
	5		0.59 ± 0.01^{a}	0.61 ± 0.01^{ab}	0.64 ± 0.02^{bc}

Values are mean ± S.D. of three independent determinations.
[a,b,c,d,e,f,g,h,i,j] Means followed by different letters are significantly different ($p \leq 0.05$).

added with chitosan. The developed protein network restricted the interference effect of chitosan. Moreover, chitosan in rice flour noodles retarded the textural change in noodles during storage. Consequently, chitosan used in flat rice flour noodles exhibits no negative effect on the rice noodle quality. However, the use of chitosan in rice starch based product should be considered carefully because such products might soften as chitosan interrupts the starch network formation.

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