

## Effect of duck egg white addition on textural properties and microstructure of rice noodles

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### Abstract

The textural properties and microstructure of freshly cooked rice noodles with different fractions of duck egg white (0-50% w/w) were investigated. The tensile strength and firmness of rice noodle increased with increasing egg white proportion. The addition of egg white 10-30% increased the breaking distance (extensibility), however, higher proportion of egg white (40 and 50%) decreased the extensibility of the noodles. The microstructure of noodle with 0% egg white revealed smooth and compact structure of gelatinized rice starch granules under scanning electron microscope. Addition of egg white resulted in rough and less compact structure of mixed rice starch-egg white protein gels. The continuity of protein matrix observed in noodles with 30% egg white indicated the net work of rice proteins and egg white proteins, distinguished by FITC and Rhodamine B staining, as illustrated under confocal laser scanning microscope. Protein matrix provides an explanation for increasing strength of rice noodles with added egg white.

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### **Introduction**

Rice flour is a principal ingredient of different types of rice noodles. The popular rice noodles in Thailand are sen yai, sen lek and sen mee (rice vermicelli) which are soft in texture and provide mainly carbohydrate. Addition of proteins from different sources to rice flour can strengthen starch gels and increase nutritional content of rice noodles. Brownsey *et al.* (1989) reported that in composite starch-protein gels, the gelatinized granules increase gel modulus at low protein concentrations whereas the inclusion of the gelatinized granules decrease the hardness of the rigid matrix gel. This is supported by the study of Noisuwan (2009) who reported the increase of final viscosity of rice starch with the addition of whey protein. The study of mixed pea starch-egg white protein gel by Brownsey *et al.* (1989) indicated that the immiscible amylose rich and protein rich phases are formed which affect mechanical behavior of the composite gel.

Duck egg yolks are widely used in some Thai desserts leaving egg white as by-product. Rapid loss of egg freshness during room temperature ( $28 \pm 3^\circ\text{C}$ ) storage was reported by Garnjanagoonchorn *et al.* (2010) as evident by the thinning of egg albumen shown by the low haugh unit (40.7) during 11 days storage. It was also observed that egg white films made from 4-day-old eggs appeared smooth and homogeneous conforming to good quality proteins. The utilization of duck egg white as a protein source

is an interesting topic. Thus in the present study egg white prepared from no later than 3-day-old duck eggs was used as a source of supplementary protein in rice noodle. The addition of different proportions of duck egg white to rice flour which affected texture, nutritional value and microstructure of rice noodles (sen yai) were examined.

### **Materials and Methods**

#### *Preparation of egg white solutions*

Fresh duck eggs no later than 3-day-old after laying, obtained from a local farm, were cleaned and separated from yolk then filtered gently two times through cheese cloth avoiding foam formation. The egg white solution was kept in a closed plastic container at  $4^\circ\text{C}$  and used within 2 days.

#### *Flour preparation*

Commercial rice flour (20 kg, Erawan brand), purchased from a local market, was mixed together then passed through a 100-mesh sieve, packed in polyethylene plastic bags and stored in plastic boxes until used.

#### *Noodle preparation*

Rice noodles containing different levels of duck egg white were prepared according to the formulation in Table 1. Rice flour, water and egg white were mixed to form rice slurries (30% w/w of rice flour) and left at room temperature ( $28 \pm 2^\circ\text{C}$ ) for 3 h. The

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Table 1. Formulation of rice noodles with 0-50% egg white

Sample code	Rice flour (g)	Water(g)	Egg white (EW)	
			wet weight (g)	dry weight (g)
EW-0	30	70	0	0.00
EW-10	30	60	10	1.29
EW-20	30	50	20	2.57
EW-30	30	40	30	3.86
EW-40	30	30	40	5.14
EW-50	30	20	50	6.43

slurries (55 g) were then poured into stainless steel trays (17.5 cm x 24 cm) and steamed at 95-100°C for 4 min. The cooking time of the rice noodle was determined as the time required for disappearance of opaque central core when squeezed gently between two glass plates. The cooked noodle sheets were cooled at ambient temperature before cutting into strips 1.5 cm x 17.5 cm (sen yai) and kept in closed containers for analyses. Commercial cooked noodles were also bought from local market early in the morning on the day of the experiment.

#### Chemical composition analysis

Proximate composition of duck egg white, rice flour and cooked noodles were determined according to AOAC (2000): moisture by the oven method; ash by the muffle furnace method; crude protein by Kjeldahl method (using 6.25 as conversion factor for egg white, cooked noodles and 5.95 for rice flour); crude fat by petroleum ether extraction method and carbohydrate by subtracting percentage of other contents (ash+fat+protein+moisture) from 100. The apparent amylose content of rice flour was determined by the colorimetric measurement of the iodine binding capacity of amylose according to the method of Juliano *et al.* (1971). All chemical analyses were reported as the means of two replicates with at least 2 samples per replicate.

#### Texture analysis

For tensile and compression tests, the cooked noodles were covered with plastic sheet and texture analysis was carried out within 15 min to avoid rapid textural changes. Texture Analyzer TA-XT2 (Stable Micro System, USA) equipped with a Spaghetti tensile grips code A/SPR was used to determine the tensile strength (peak force, g) and extensibility (peak distance, mm). A strand of cooked noodle was wound two or three times around parallel friction roller of the grip to anchor the samples and avoid slippage. The mode was measure force in tension. The upper arm was set to travel apart from the lower arm at a speed of 3.0 mm/s. The peak force (g) required to break the noodle strand gave an indication of the sample resistance to the breakdown and the distance (mm) in which the strand started to break indicated the extensibility. For compression test, Texture

Analyzer equipped with Light knife blade probe code A/LKB was used to measure the hardness (peak force of cutting, g) and the total work of cutting (g-s). The mode was measure force in compression with single cycle and test speed of 0.2 mm/s. A strand of cooked noodle with 1.0 mm thickness was put on the platform and tested. Two replicates were carried out using 15 samples for each treatment.

#### Analysis of microstructure of cooked noodles

Microstructure of cooked noodles with added egg white at 0, 30, 50% (EW0, EW30, EW50) were investigated using Scanning electron microscope (SEM, Hitachi SU 1500, Japan) and noodle with 30% egg white was examined using Confocal laser scanning microscope (CLSM, ZEISS LSM5 PASCAL, Germany).

For SEM, cooked noodles were gradually dehydrated in graded series of 50, 70, 90 and 100% ethanol at room temperature (28±2°C) for 24 h at each concentration then dried in a critical point dryer. The samples were fractured to obtain cross-section of the noodle, before mounted on an aluminum specimen stub with the cross-section face up. Finally the samples were sputtered with gold and observed under a SEM and detecting secondary electron. The images were provided at an accelerating voltage of 15.0 kV at various magnifications.

For CLSM, freshly prepared noodles (EW30) were cut into 5x5 mm pieces and double staining was performed to visualize protein and carbohydrate component in noodle microstructure. Samples were stained with fluorescein isothiocyanate or FITC (0.01% w/v in dimethyl sulfoxide) for 30 s, rinsed with distilled water to remove excess dye followed by Rhodamine B (0.01% w/v in dimethyl sulfoxide) staining for 30 s and rinsed with distilled water. Samples were placed on glass slides longitudinal section face up and immersed in glycerol then covered with cover slips. Samples were examined under CLSM (Model LSM5 PASCAL, ZEISS, Germany) equipped with Helium-Neon laser where FITC and Rhodamine B were excited at 488 and 543 nm respectively and emitted light at 500-530 and 620-650 nm, respectively.

In addition, the noodle samples were pre-stained with two fluorescent dyes, FITC and Rhodamine B. FITC solution (0.01%w/v in dimethyl sulfoxide, 50 µL) was mixed into 5 g of egg white and stained for 3 h while Rhodamine B solution (0.01%w/v in dimethyl sulfoxide, 100 µL) was mixed into the rice flour slurry 11.67 g (30% w/w), and stained for 3 h. The pre-stained egg white and rice flour slurry were mixed together, spread on a plate and steamed

as described previously. Another cooked noodle sample was prepared from pre-stained egg white and unstained rice flour slurry. The cooked noodles were then cross-sectioned and observed under CLSM.

*Statistical analysis*

Rice noodles and all data analyses were performed in duplicates. All data were subjected to analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) to compare treatment means; differences were considered at significant level of 95% (p < 0.05) (SPSS version 12 software).

**Results and Discussion**

*Chemical composition analysis*

Proximate composition of rice flour and duck egg white are presented in Table 2. The moisture content was 12.79 and 87.14% (w/w, wet basis) for rice flour and egg white, respectively. Rice flour contains high carbohydrate (90.29% dry basis) with high amylose content (32.04% dry basis) and low protein (9.19% dry basis) whereas egg white consists mainly of protein (94.26% dry basis) and thus is suitable as protein supplement in rice flour. Analysis of rice noodle containing 0 and 30% fresh egg white showed protein content of 8.32 and 16.07% (w/w, dry basis), respectively. The addition of fresh egg white increased the protein content thus resulted in better nutritional value of the noodles.

*Effect of egg white on textural properties of rice noodle*

Textural properties of rice noodles containing 0, 10, 20, 30, 40, 50% (w/w) egg white were investigated (Table 3). The results indicated that tensile strength and firmness of rice noodle increased with increasing proportion of egg white. Addition of egg white 10-30% increased the breaking distance (extensibility), however, higher proportion (40 and 50%) decreased the extensibility of the noodles and increased rough appearance on noodle surface (Figure 1). Commercial rice noodles which are more transparent than the experimental noodles were shown to have the highest breaking distance with low tensile strength and firmness (Figure 1). It is likely that other ingredients used in commercial rice noodles, like tapioca starch and modified starch, were responsible for the increasing of glass like appearance. Egg-white proteins function by forming thermally induced gels while starch polymer gels on cooling. Malcolmson *et al.* (1993) also reported that the firmness of spaghetti is related to protein content as the higher amount of protein to interact and form

Table 2. Proximate composition of rice flour and fresh duck egg white

Composition	Rice flour		Duck egg white	
	%wet basis (w/w)	%dry basis (w/w)	%wet basis (w/w)	%dry basis (w/w)
Moisture <sup>1</sup>	12.79 ± 0.20	-	87.14 ± 0.01	-
Protein	8.02 ± 0.22	9.19 ± 0.27	12.55 ± 0.55	94.26 ± 4.13
Fat	0.20 ± 0.03	0.23 ± 0.04	0.05 ± 0.02	0.06 ± 0.02
Ash	0.26 ± 0.00	0.29 ± 0.00	0.68 ± 0.01	5.42 ± 0.04
Carbohydrate	78.73 ± 0.26	90.29 ± 0.32	trace	trace
Amylose <sup>1</sup>	-	32.04	-	-

<sup>1</sup> - - - = No report

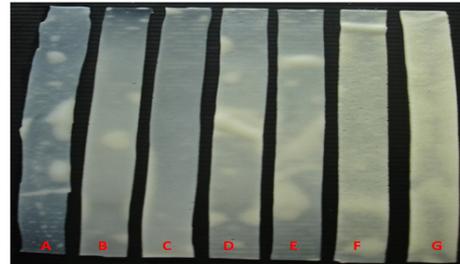


Figure 1. Fresh rice noodle: (A) commercial rice noodle; (B) rice noodle with 0% egg white; (C), (D), (E), (F) and (G) rice noodles with 10, 20, 30, 40, and 50% egg white, respectively.

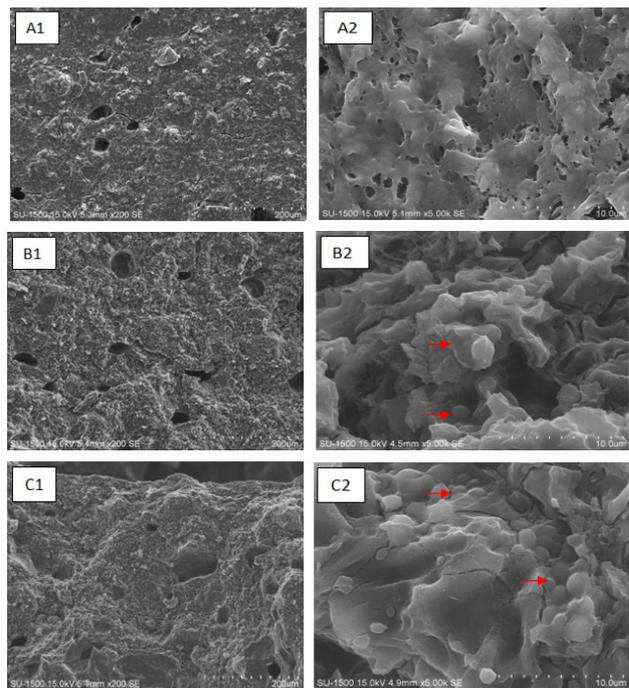


Figure 2. Scanning electron micrographs of cross-sections of cooked rice noodles containing 0, 30 and 50% egg white: (A) 0% egg white; (B) 30% egg white; (C) 50% egg white. Arrows indicate zone of aggregation or strand structure. 1 and 2 indicate low magnification and higher magnification. Accelerating voltage and scale bars are included in the micrographs.

network the more firmness of pasta obtained. In the present study higher proportion of egg white (40 and 50%) resulted in the formation of rigid and fragile gel. These textural data indicated that by adjusting the proportion of rice flour and egg white protein content in the formulation the desired noodle texture

can be formed.

#### Microstructure of rice noodles with added egg white observed by SEM and CLSM

Microstructure of cooked rice noodles, EW0, EW30 and EW50 were observed by SEM. The microstructure of noodles changed with increasing egg white concentrations. EW0 appeared as smooth and compact structure of gelatinized starch granules (Figure 2-A1) while EW30 and EW50 displayed rough and less compact structure with increasing number and size of pores (Figure 2-B1 and 2-C1). Brownsey *et al.* (1989) observed that in starch-egg-white protein gel the protein and amylose are immiscible on a microscopic scale and are distributed unevenly throughout the gel. Previous studies also illustrated that microstructure of protein gels were rough and consisted of denser areas of aggregated protein structure and pores throughout the network, where pores could be trapped water (Gomez-Guillen *et al.*, 1997; Aryana *et al.*, 2002; Tammattinna *et al.*, 2007; Rawdkuen and Benjakul, 2008).

Micrographs at higher magnification (Figure 2-A2, 2- B2 and 2-C2) revealed the gel network of rice noodle with 0% egg white (EW0) presented differently from the egg white added noodles (EW30 and EW50). The noodles with added egg white consisted of strands of aggregated structure (indicated by red arrows) embedded in starch gel network. The area of granular aggregated structure could probably be the structure of egg white protein matrix as it increased with increasing proportion of egg white in the rice noodles (Figure 2-B2 and 2- C2). Increasing of protein fraction in rice flour provides the continuity of protein matrix which affects the mechanical strength of the noodles (Saini, 2007). Thus in this study EW50 showed higher tensile strength and maximum cutting force than EW30 and EW0 (Table 3). In order to locate the egg white protein structure and starch gel network the EW30 noodles were prepared by pre-and post-staining with FITC and Rhodamine B and observed under CLSM (Figure 3). CLSM micrographs indicated that egg white proteins, stained by FITC (Tromp *et al.*, 2003), connected and formed protein matrix as seen in green color, while rice proteins, stained by Rhodamine B, were shown as bright orange-red color structure and starch gel stained by Rhodamine B was seen in dull orange color (Figure 3A). However pre-staining of egg white with FITC and starch with Rhodamine B before formulated to rice noodles showed starch gel in dull green color (Figure 3B) while pre-staining of egg white alone with FITC showed protein in bright green color and starch gel

Table 3. Textural properties of cooked rice noodle with different proportion of egg white

sample	Tensile test <sup>1</sup>		Cutting test <sup>1</sup>	
	Peak force (g)	Peak distance (mm)	Peak force (g)	Total work (g.s)
EW0	60.22 ± 4.62b	23.31 ± 1.70a	315.82 ± 7.99a	1768.86 ± 384.77a
EW10	74.10 ± 1.75c	25.77 ± 1.08bc	407.47 ± 27.20b	2043.08 ± 195.55a
EW20	85.02 ± 2.72cd	25.97 ± 0.62bc	484.26 ± 23.30c	2371.97 ± 236.82a
EW30	83.97 ± 4.36cd	26.83 ± 0.99c	634.98 ± 11.01d	3171.20 ± 48.92 b
EW40	93.71 ± 11.97de	24.37 ± 0.52ab	766.74 ± 45.03e	4035.51 ± 611.89 c
EW50	100.91 ± 4.72e	23.27 ± 0.37a	837.25 ± 25.90f	4415.33 ± 86.58 c
commercial	46.75 ± 0.08a	34.10 ± 0.42d	417.83 ± 13.39b	2193.82 ± 225.11a

<sup>1</sup>means with different letters (a-f) within the same column are significantly different  $p < 0.05$

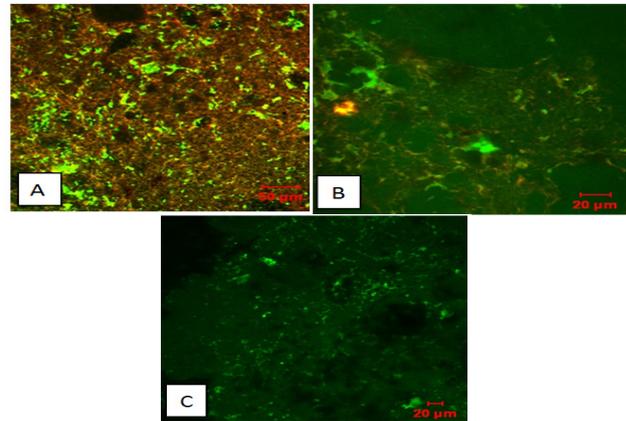


Figure 3. Confocal laser scanning micrographs of rice noodle with 30% added egg white (EW30).

(A) L-section, post-staining of cooked noodle with FITC followed by Rhodamine B: bright green color represents egg white protein, bright orange-red color represents rice protein and dull orange color represents rice starch gel; (B) X-section, pre-staining of egg white with FITC and rice flour slurry with Rhodamine B: bright green color represents egg white protein, bright orange color represents rice protein and dull green color represents rice starch gel; (C) X-section of pre-staining sample, FITC stained egg white mixed with rice flour slurry: bright green color represents protein and dull green color represents rice starch gel. Scale bars are included in the bottom right of each micrograph.

in dull green color (Figure 3C). According to Tromp *et al.* (2003) FITC and Rhodamine B can both be used for non-covalent staining of proteins whereas in this study double staining with both dyes indicated that FITC stained water soluble egg white protein while Rhodamine B stained rice protein located at the outer part of starch granules and granule remnant then stained rice starch/starch gel. The pre-staining noodle micrographs indicated starch gel structure in dull green color which possibly because steaming process destroyed Rhodamine B-starch attachment while retaining FITC-starch linkage. Both SEM and CLSM micrographs confirmed that starch and protein networks were immiscible however increases of continuous protein matrix could provide strength for rice noodle.

#### Conclusion

The microstructure of freshly cooked rice noodle changed with the addition of duck egg white. The smooth and compact structure was transformed to rough and less compact with increasing continuity of protein network when the proportion of duck egg white increased. The continuity of protein matrix was responsible for the changes in mechanical properties of the rice noodles. The protein content of the rice

noodle or sen yai (in Thai) containing 30% egg white was two times that of the noodle without added egg white.

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