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Morphological and Functional Properties of Gluten-free Rice Spaghetti based on Rice Flour, Egg White Protein Powder and Soy Protein Isolate

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ABSTRACT

The aim of this study was to determine the effect of egg white protein powder and soy protein isolate on the morphological and functional properties, and consumer acceptance of gluten-free rice spaghetti (GFRS). GFRS made from dry-milled high amylose rice flour (Chai Nat 1) which was blended with dry-milled waxy rice flour (RD6) at a ratio of 90:10 (w/w) with egg white protein powder (EWPP) varying at 0, 2.5, 5.0% db and soy protein isolate (SPI) varying at 0, 2.5, 5.0% db. The GFRS formulations were processed using a co-rotating twin-screw extruder up to 95 °C and then dried at 40 °C. The GFRS samples were analyzed by fluorescence microscopy, scanning electron microscopy (SEM), texture analyzer and sensory evaluation. Results showed that the fluorescence micrograph of the cross section of the GFRS containing 2.5% EWPP and 5.0% SPI (GFRS-E2.5S5) had a uniformly distributed protein network. SEM indicated that the surface of GFRS-E2.5S5 contained small pores of consistent size. The GFRS-E2.5S5 had the highest value of firmness and tensile strength, and the lowest stickiness. The consumer acceptance of the cooked GFRS samples was evaluated on the 9-point Hedonic scale. The result showed that GFRS-E2.5S5 had the highest score of overall acceptability (6.20) among all GFRS samples.

INTRODUCTION

Spaghetti is one of the most consumed foods in the world. Spaghetti is traditionally made from durum wheat (Triticum durum L.) semolina and water kneaded together to form dough. Then the dough is processed by extrusion into a round shape. Gluten is an important protein in wheat flour and has functional properties in spaghetti [1]. However, gluten is a trigger of Celiac disease and inflammatory disorders of the small intestine, which can lead to malabsorption. A report has shown that Celiac disease has a prevalence in the United States and Europe of approximately 1% in the general population [2]. Gluten-free products made from rice could be alternative products for Celiac patients [3].

In our previous work on gluten-free rice spaghetti (GFRS), which is made from rice flour with intermediate to high amylose. The percentage of amylose is important in rice spaghetti, because it is involved in starch network. Detchewa et al (2012) studied the effects of differences in amylose content by adjusting the ratio of dry-milled high amylose rice flour (Chai Nat 1) and dry-milled waxy rice flour (RD 6) [4]. The results showed that an amylose content of 29% was suitable for making GFRS due to high firmness and tensile strength. However, the quality of GFRS is still lower than that of spaghetti produced from durum wheat semolina.

Furthermore, the development of gluten-free rice spaghetti was done by adding proteins. Protein is a desirable food ingredient due to its nutritional value and its effect on the texture of food products. Egg white protein is considered to be excellent for its nutritional and function attributes (foaming, emulsifying and gelation). Ovalbumin is the main protein in egg white (54%) and has free sulfhydryl (SH) groups which enable it to form a gelation [5]. Soy protein is widely used in the food industry. The major soybean proteins are ß-conglycinin and glycinin. Soy protein is an excellent gelation agent and plays a role in forming cohesive structure [5]. Detchewa and Naivikul (2016) made GFRS from dry-milled rice flour with egg white protein powder (EWPP). The addition of EWPP at 5% into GFRS improved texture and sensory attributes due to modification of the food matrix [7]. Detchewa et al. (2016) developed GFRS by adding soy protein isolate (SPI). The addition of SPI at 5% in GFRS improved cooking quality, texture parameters and sensory evaluation. Scanning electron microscopy showed that the surface of the GFRS with SPI was more porous than GFRS without SPI[8].

Therefore, the aim of this study was to investigate the effect of egg white protein powder and soy protein isolate on the morphological properties, molecular size distribution, cooking quality, texture parameters and sensory evaluation of gluten free rice spaghetti as compared to commercial wheat spaghetti.

METHODOLOGY

Two types of dry-milled rice flour were prepared from two rice varieties (*Oryza sativa* L.): high amylose rice, Chai Nat 1 (CNT1) and waxy rice, Rice Divison 6 (RD6) by the following method of Detchewa et al. (2012). Egg white protein powder (EWPP) and soy protein isolate (SPI) were purchased from Winner Group Enterprise and Vicchi Enterprise Co., Ltd. Thailand. Commercial wheat spaghetti (Spaghetti Ristorante 8, F. Divella S.p.A., Italy) was purchased from a local market.

Production of gluten-free rice spaghetti

The blended dry-milled rice flour was mixed with egg white protein and soy protein isolate as shown in Table 1. Each rice flour formulation was mixed using a cubic mixer and extruded using a co-rotation twin-screw extruder (model EVO25 A120, Clextral, France) up to 95 ^{o}C with screw speed of 220 rpm, as described method by Detchewa et al. (2016) [8].

Table 1 . Nomenclature of gluten-free rice spaghetti (GFRS) and proportion of rice flour, egg white protein powder (EWPP) and soy protein isolate (SPI)

Nomenclature	CNT 1 (%)	RD 6 (%)	EWPP (%)	SPI (%)
Commercial wheat spaghetti	-	-	-	-
GFRS-0	90.00	10.00	0.00	0.00
GFRS-E2.5S2.5	85.50	9.50	2.50	2.50
GFRS-E2.5S5	83.25	9.25	2.50	5.00
GFRS-E5S2.5	83.25	9.25	5.00	2.50
GFRS-E5S5	81.00	9.00	5.00	5.00

Morphological analyses

Fluorescence microscopy

The GFRS and commercial wheat spaghetti samples were soaked in water for 4 h. Then, the water was separated from the samples by sieving. The spaghetti samples were frozen with liquid carbon dioxide and cut into 9 um thick sections using a cryostat (Leica CM 1850-3-1) at -20 ^{o}C [9]. The cross-section samples were mounted on microscope slides and were stained for protein with Rodamine B, 0.01 g/L, and examined under a fluorescence microscope (Zeiss axioplan, Germany).

Scanning electron microscopy

The surface of the sample was examined using a scanning electron microscope (SEM). Each sample was mounted on stubs and coated with gold and was observed using an SEM (JEOL JSM-5300 LV SEM, USA) at 1000 x magnification.

Molecular size distributions using gel-permeation chromatography (GPC)

The molecular size distributions of the samples were determined by sepharose CL-2B gel permeation chromatography (GPC), following the method of Zweifel et al. (2003) [10]. Molecular size distributions were determined on the basis of total carbohydrate (anthrone–sulfuric acid reaction) measured at 530 nm. Blue values were used to identify the locations of amylose and amylopectin in the chromatograms at 640 nm.

Cooking qualities and texture analyses

The cooking qualities of the samples were determined by the cooking time, cooking loss and water absorption index, following the method of Detchewa et al. (2016) [8] and AACC (2010) [8]. The samples were cooked for the optimal time, after which the texture parameters of the samples were determined. Firmness, tensile strength and stickiness were measured using a texture analyser (TA-XT plus, Stable Micro System, Godalming, England). The method was previously described in Detchewa et al. (2016) [8].

Sensory evaluation

The samples were cooked for the optimal time and served to 30 panelists. The panelists evaluated the samples for appearance, color, flavor, texture, and overall acceptability on a 9-point hedonic scale, where 9=extremely like and 1= extremely dislike.

Statistical analysis

All experiments were carried out in duplicate. The mean and standard deviation of the parameters were calculated and the difference between the samples were evaluated by analysis of variance (ANOVA) using SPSS. Duncan's Multiple Range Test was used to analyze the difference between samples; samples were declared different at $p<0.05$.

RESULTS AND DISCUSSION

Fluorescence microscopy

The fluorescent micrographs of commercial durum wheat spaghetti showed a continuous-phase gluten network (red color) (Figure 1A). Under the polarized light, the wheat starch granules exhibited the Maltese cross effect, which indicates that the starches were not gelatinized. The spaghetti extrusion was conducted under controlled temperature below 50 ºC [12], and thus the starches in the wheat were not gelatinized prior to cooking.

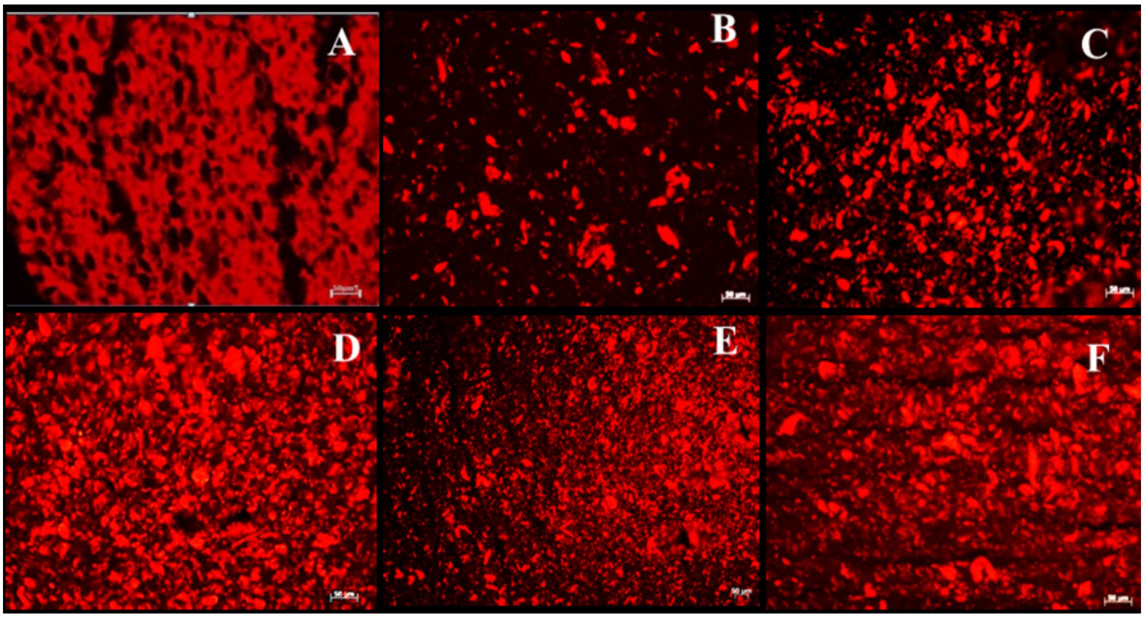

Figure 1 Fluorescent micrograph of cross sections of durum wheat spaghetti and GFRS with EWPP and SPI. Commercial durum wheat spaghetti (A)* and GFRS-0 (B)*, GFRS-E2.5S2.5 (C), GFRS-E2.5S5 (D), GFRS-E5S2.5 (E), and GFRS-E5S5 (F)
*From Detchewa et al. (2016) [8].

The GFRS-0 showed a sparsely-dispersed protein-rich phase located randomly in the rice spaghetti; the mixing was not homogenous (Figure 1B). The GFRS-0 did not show the Maltese cross under light microscope with polarized light. Most probably, the starch in the rice flour was cooked during extrusion at high temperature (95 °C) and high speed screw (220 rpm), resulting in fully gelatinized starch.

In the case of the 2.5% EWPP and 5.0% SPI (GFRS-E2.5S5), the micrograph showed that the protein-rich phase was uniformly distributed and was able to form a network. This structure can affect the texture and sensory evaluation. When increasing the total protein content to 10% (EWPP 5% and 5%SPI, GFRS-E5S5), the micrograph showed an even but not homogenous protein phase distribution throughout the strand, as the micrograph showed large aggregates of uneven sizes of the protein-rich phase that were not well distributed.

Scanning electron microscopy

Scanning electron micrographs (SEM) of the surface of the durum wheat semolina and GFRS samples are shown in Figure 2. The surface of the commercial durum wheat spaghetti had a more porous structure and starches that were embedded in the continuous protein matrix. The micrographs of GFRS-0, GFRS-E2.5S2.5, GFRS-E2.5S5, GFRS-E5S2.5 and GFRS-E5S5 are shown in Figure 2 B, C, D E, and F, respectively. The GFRS-E2.5S5 exhibited small pores throughout the strand, while GFRS-E5S5 had a rough surface. Thus, the GFRS-E2.5S5 had a smoother surface than the other GFRS. This result was similar to the effect of adding SPI in a previous experiment, which showed an increase in surface porosity with increasing SPI content level. GFRS-E2.5S5 was observed to have small pores of consistent sizes.

Molecular size distributions using gel-permeation chromatography (GPC)

The extrusion process affected the molecular structures of the starch molecules, which are composed of amylopectin and amylose. The molecular weight distribution of GPRS using gel permeation chromatography was investigated. The results showed that amylopectin with a large molecular weight was eluted at void volume and shown in the first peak in Figure 3.

The second peak in Figure 3 represented amylose molecules and might include amylopectin degradation and amylose retrogradation in the same fraction. The percent of amylopectin was calculated from GPC [10]. The commercial spaghetti contained 55.42% amylopectin, which was higher than the rice spaghetti samples. The commercial spaghetti GPC profile was similar to wheat starch because the processing of the commercial durum wheat spaghetti did not involve high heat during extrusion. The extrusion process could induce amylopectin degradation, causing an increase in the second peak. Results showed that the percent of amylopectin in GFRS-0, GFRS-E2.5S2.5, GFRS-E2.5S5, GFRS-E5S2.5, and GFRS-E5S5 were 42.56%, 44.51 %, 44.86 %, 44.19 %, and 45.04 %, respectively. All GFRS samples that contained EWPP and SPI showed significant difference ($p<0.05$) from GFRS-0. Adding proteins in rice spaghetti samples led to retardation of starch degradation.

Cooking qualities and texture analyses

GFRS cooking quality parameters are shown in Table 2. There were significant differences in optimum cooking time, cooking loss, and water absorption index. GFRS with EWPP and SPI levels showed

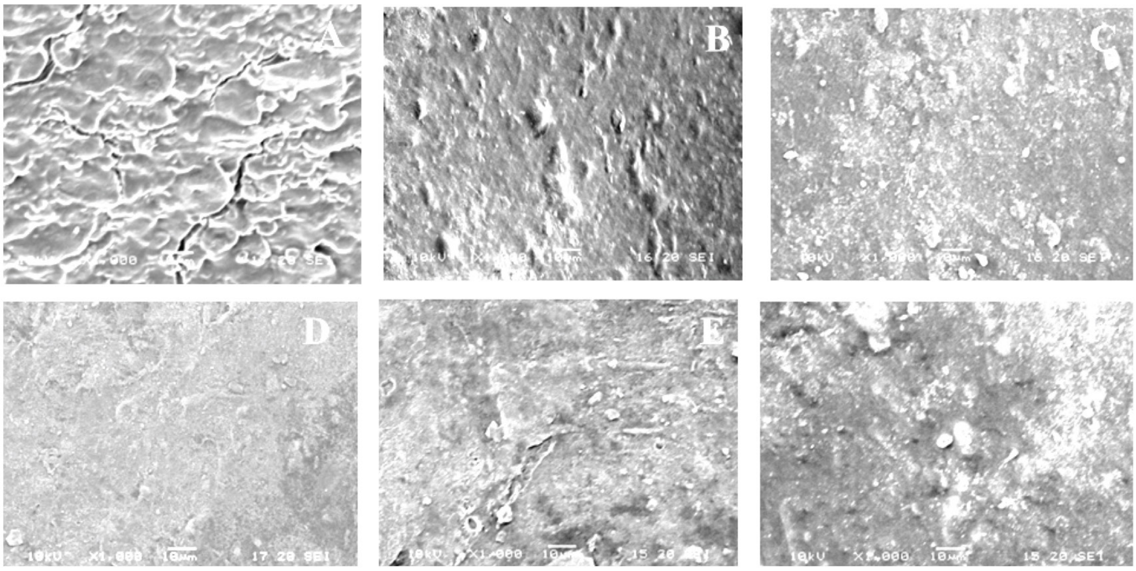

Figure 2 Scanning electron microscope of surface of commercial durum wheat spaghetti and GFRS with EWPP and SPI. Commercial durum wheat spaghetti (A)*, GFRS-0 (B)*, GFRS-E2.5S2.5(C), GFRS-E2.5S5(D), GFRS-E5S2.5(E), and GFRS-E5S5(F). *From Detchewa et al. (2016) [8].

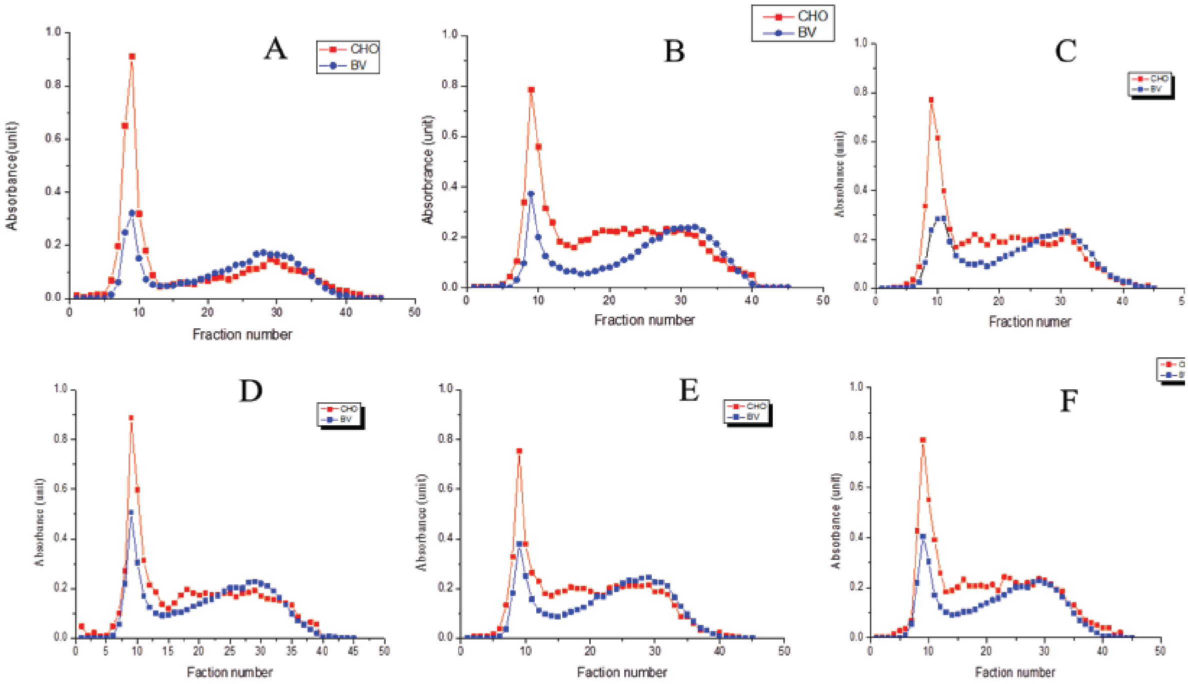

Figure 3 Sepharose CL-2B gel permeation chromatography profiles of commercial durum wheat spaghetti and GFRS with EWPP and SPI: Commercial durum wheat spaghetti (A), GFRS-0 (B), GFRS-E2.5S2.5(C), GFRS-E2.5S5(D), GFRS-E5S2.5(E), and GFRS-E5S5(F). CHO: total carbohydrate content, BV: blue value.

a shorter optimum cooking time than GFRS-0. The addition of 2.5% EW and 5.0% SPI (GFRS-E2.5S5) decreased cooking time from 17.66 min to 13.33 min.

Commercial durum wheat spaghetti showed the shortest optimum cooking time (12.10 min). The microstructure of the commercial durum wheat spaghetti (Figure 2A) showed small holes. These results are similar to those of Wang et al. (1999) [13] who found that wheat spaghetti had a lower cooking time than gluten-free pea pasta. The wheat spaghetti showed some cracks and small pores on its surface which allowed water access during cooking.

The cooked weight of GFRS-0 was 246.25 g/100 g. The cooked weight increased with increasing EWPP and SPI contents. The water adsorption indices of GFRS-E2.5S2.5, GFRS-E2.5S5, GFRS-E5S2.5 and GFRS-E5S5 were lower than the commercial durum wheat

Table 2 Effect of EWPP and SPI on the cooking quality of GFRS samples and commercial durum wheat spaghetti

Samples	Cooking time(min)	Cooking Loss (%)	Water Absorption index (%)
Commercial wheat spaghetti*	12.10 ± 0.17d	6.83 ± 0.28f	298.33 ± 1.52a
GFRS-0*	17.66 ± 0.70a	25.49 ± 0.75a	246.40 ± 4.20c
GFRS-E2.5S2.5	15.67 ± 0.57b	20.53 ± 0.65c	241.66 ± 2.88cd
GFRS-E2.5S5	13.33 ± 0.60c	13.70 ± 0.28e	252.70 ± 2.51b
GFRS-E5S2.5	13.50 ± 0.50c	15.56 ± 0.46d	237.10 ± 2.64d
GFRS-E5S5	13.60 ± 0.60c	22.29 ± 0.24b	238.10 ± 3.10d

Values are means of measurements ± standard deviation. Mean for each characteristics followed by the different letter within the same column are significantly different ($p<0.05$).*From Detchewa et al. (2016) [8].

Table 3 Effect of EWPP and SPI on the texture parameters of cooked GFRS samples and commercial durum wheat spaghetti

Samples	Firmness (N)	Tensile strength (N)	Stickiness (N)
Commercial wheat spaghetti*	3.85 ± 0.07a	0.30 ± 0.01a	0.10 ± 0.03c
GFRS-0*	2.23 ± 0.05c	0.07 ± 0.05d	0.40 ± 0.03b
GFRS-E2.5S2.5	2.24 ± 0.12c	0.12 ± 0.01c	0.33 ± 0.06b
GFRS-E2.5S5	2.55 ± 0.17b	0.16 ± 0.01b	0.14 ± 0.02c
GFRS-E5S2.5	2.20 ± 0.16c	0.10 ± 0.02d	0.39 ± 0.01b
GFRS-E5S5	1.91 ± 0.26d	0.08 ± 0.01d	0.42 ± 0.01a

Values are means of measurements ± standard deviation. Mean for each characteristics followed by the different letter within the same column are significantly different ($p<0.05$). *From Detchewa et al. (2016) [8].

spaghetti. The water adsorption index indicated the swelling ability of starch and protein [14,15]. Cooking loss was an important parameter related to consumer acceptance in a pasta product [16]. The addition of 2.5%EWPP and 5.0 % SPI caused a reduction in cooking loss from 25.49 % to 13.70 %. However, the GFRS samples had higher cooking loss than the commercial durum wheat spaghetti.

Firmness, tensile strength, and stickiness that are important textural attributes for spaghetti were determined. Results showed that the firmness and tensile strength of the commercial wheat spaghetti were the highest value, but stickiness was the lowest value (Table 3). The firmness and tensile strength were linked to the internal structure of the spaghetti. Stickiness was related to the surface properties of the cooked spaghetti. The fluorescence micrograph (Figure 1) showed the presence of a continuous gluten network, which gave the spaghetti high firmness, tensile strength and low stickiness.

The GFRS-E2.5S5 showed the highest firmness, tensile strength, and lowest stickiness among the GFRS samples. The texture parameters were related to protein and starch matrix as shown in the fluorescence micrographs (Figure 1). The GFRS-E5S5 (10% protein content) showed decreased firmness and tensile strength which might be due to the protein interfering with starch gel.

Sensory evaluation

Sensory evaluation of the GFRS samples and commercial wheat spaghetti was determined in terms of appearance, color, flavor, texture and overall acceptability as shown in Table 4. The GFRS without added protein (GFRS-0) received the acceptability score of 5.26, indicating a “neither like nor dislike”, while the GFRS-E2.5S5 received the highest overall acceptability score of 6.20, indicating a “slightly like to moderately like”. However, the commercial wheat spaghetti received the highest score for all sensory parameters.

CONCLUSION

Gluten-free rice spaghetti was made from dry-milled rice flour with EWPP and SPI using a twin-screw extruder. The addition of 2.5% EWPP and 5% SPI improved the cooking qualities, texture parameters and sensory attributes of GFRS. The fluorescence micrograph of GFRS-E2.5S5 revealed a uniformly distributed protein network, which resulted in an increase in the firmness and tensile strength, and a decrease in the stickiness. The scanning electron micrograph of GFRS-E2.5S5 showed small pores of consistent size, resulting in a decrease in cooking time and cooking loss. Sensory evaluation of the cooked samples was performed on a 9-point hedonic scale. The GFRS-E2.5S5 had the highest score for overall acceptability (6.20, indicating “slightly like to moderately like”).

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