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REGULAR ARTICLE

Process optimization to increase resistant starch in vermicelli prepared from mung bean and cowpea starch

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ABSTRACT

This research developed a process to increase the resistant starch (RS) content in vermicelli prepared from a mixture of cowpea and mung bean starch. The physicochemical and functional properties of mung bean starch (M) and cowpea starch (C) were evaluated at different ratios to determine the optimum starch mixture for the vermicelli. A response surface method (RSM) with central composite design (CCD) was used to determine the effect of incubation times at 4°C, and freezing times at -13°C on the RS content in the vermicelli. The results suggested that the starch mixture of M:C (50:50) was the most suitable for preparing vermicelli. The optimum condition to improve the RS content of the vermicelli using RSM was incubation of the fresh noodles at 4°C for 1 h 30 min, followed by freezing at -13°C for 21 h (R^2 = 0.95). Under these conditions the RS content of the uncooked noodles increased to 29.20%, compared with vermicelli prepared without incubation (20%) and by the reference method (23.93%).The cooking and texture qualities of the vermicelli were also evaluated. The optimization of RS content had no effect on the cooking and texture quality of the noodles, and statistical tests indicated that the RSM model showed good fit and was suitable for application in high resistant starch vermicelli production.

Keywords: Cowpea; Mung bean; Noodles; Resistant starch; Vermicelli

INTRODUCTION

The modern trend is toward healthier food consumption. Foods with high resistant starch (RS) have become popular as RS is not easily digested, and has the positive benefits of improving insulin sensitivity and reducing blood sugar levels in the digestive system (Reader et al., 1997). In the human body, undigested RS passes through to the colon and undergoes fermentation which forms pro-biotic bacteria that are beneficial for health (Brown, 1996). Nowadays, RS is used as a food ingredient for healthy food because of its benefit as a dietary fiber. There are four types of RS as RS1, RS2, RS3, and RS4. RS1 and RS2 are naturally found in starchy foods, but only in small amounts and they are not stable when processed. RS3 is a retrograded starch induced by moist heat treatment, whereas RS4 is a chemically modified starch. RS3 is safer than RS4 as it is produced by inducing the starch to retrograde, and becomes more stable when food is processed.

Vermicelli is a legume product, and it is considered a good source of RS. Vermicelli contains an RS content of between 10 and 20%. This varies depending on the production process and the raw materials used. Vatanasuchart et al. (2009) studied the RS content of Thai noodle products. They found that vermicelli had an RS content of 11.3%, higher than instant rice and rice sheet noodles (2.40%). The RS in vermicelli comes from the starch itself, and from the retrograded starch produced when the noodles are processed and subjected to a freeze-thaw cycle which variations in the cycle could increase the content of RS3 (Chung et al., 2003). Some manufacturers however do not include these processes in vermicelli manufacture, and there is wide variation in the RS content of glass noodles. The main reason for incubation and freezing was historically to assist in the drying process of the noodles. The temperatures for incubation and freezing times used were different. Chansri et al. (2005) prepared edible canna starches from clear noodles, frozen at -10°C for 24 h. Shung and Stone (2004) also prepared the noodles by freezing at -10°C for

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24 h. Tsakama et al. (2013) prepared sweet potato starch noodles by first cooling at 4°C in a refrigerator for 6 h, and then freezing at -5°C for 8 h, while Tam et al. (2004) made the noodles by first hanging to partially dry, then kept at 4°C for 2 h, followed by freezing at -10°C overnight. Galvez et al. (1994) stored starch noodles at -18 to 5°C for 12 – 24 h to increase retrogradation, while Tan et al. (2006) prepared glass noodles by storage at 4°C for 2 h, and then freezing at -13°C for 24 h. This study followed their method. Some researchers reported that the freezing process produced softer and stickier textured noodles (Korean vermicelli) (Lee et al., 2005).They suggested that to improve the firmness (or hardness) and elasticity of the noodles, the vermicelli should be incubated in cold storage at 4°C for 21 h. However, none of these studies reported on the effect of RS content from the incubation of glass noodles at low temperature and freezing. Storage at 4°C for 24 h before oven drying slightly increased of RS3 in waxy and regular barley flours (Faraj et al., 2004). Gebhardt et al. (2001) reported that cold storage at -18°C gave the best results for RS formation.

Vermicelli usually made from mung bean (*Vigna radiata* (L.) R. Wilcz). It contains high amylose content, a typical C-type viscosity pattern, high stability, and restricted swelling (Meatres et al., 1998; Ohwada et al., 2002). However, mung beans are expensive as world production is limited, resulting in low supply (Zhang and Chi, 2001). Therefore, an alternative to mung bean starch is required as a source for vermicelli. The substitution of other starches at high levels resulted in reduced eating quality with a dull opaque appearance, high cooking loss, poor elasticity, and a reduction in the glossy appearance (Hoover et al., 1997; Tan et al., 2006; Kim et al., 2007).

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most important legumes in African and Asian countries. Cowpea attracted the attention of this study through the report of Kweon et al. (1993) who discovered that cowpea starch had strong hardness and cohesiveness similar to mung bean starch. Chung et al. (1998) found that cowpea starch gel had the highest hardness, chewiness, and springiness. These properties supported the hypothesis that cowpea starch could replace mung bean starch for glass noodle production. Lee et al. (2006) suggested that a 1:1 ratio of cowpea: sweet potato starch improved texture and cooking quality similar to commercial vermicelli, and increased storage at 4°C from 3 to 21 h resulted in reduced cooking loss and increased water uptake.

Therefore, this study was carried out, replacing cowpea starch for mung bean starch in glass noodle preparation. Process optimization using response surface methods (RSM) was applied to increase the level of RS content by optimizing the incubation period at 4°C and freezing time at -13°C.

MATERIALS AND METHODS

Starch preparation

Mung bean (*Vigna radiata* (L.) R. Wilcz) and cowpea (*Vigna unguiculata* (L.) Walp) seeds were purchased from a local market in Maha Sarakham, Thailand. The starch preparation modified the method of Park et al. (2012). The seed samples (three replicates) were first cleaned, and then soaked in tap water (seeds: tap water, 1:3) at room temperature for 8 – 10 h. The seeds were then blended with tap water at low speed (100 rpm). The supernatant slurry was filtered through sieve screens (70, 100, and 200 mesh, respectively). The residue was washed with tap water until free of starch. The starch-protein slurry filtrate was passed successively over 200 mesh sieve screens and allowed to stand for 4 – 5 h. The supernatant was then removed by suction, and the settled starch layer was re-suspended in distilled water. The upper layer was scraped off and the white layer was re-suspended in distilled water and re-centrifuged 3 to 4 times. The starch was then collected and dried in an oven at 45°C for 12 h to obtain 10% moisture content (wet basis). It was then finely ground, passed through a 70 mesh sieve, packaged in plastic bags and kept in a refrigerator.

Mixtures of mung bean and cowpea starch

The starch was prepared from the mixture of mung bean (M) and cowpea (C) for five different percentages, being mung bean (100:0), 80:20, 50:50, 20:80 and cowpea (0:100).

Resistant starch (RS) determination

The RS content was analyzed using the Megazyme resistant starch assay kit (Megazyme International Ireland Ltd, Bray, Ireland).

Swelling power and solubility

Swelling power (*SP*) and solubility were determined using the modified method of Lii and Yeh (2001). The mung bean starch, cowpea starch, and starch mixture (0.1 g, dry basis) was combined with distilled water (10 mL) in a 50 mL centrifuge tube. The tube was heated in a water bath at 55, 65, 75, 85, and 95°C for 1 h, then cooled to room temperature and centrifuged (8,000×g) for 20 min. The supernatant was then removed from the tube and the sediment was weighed (*WS*). The supernatant was dried at 105°C until a constant weight (*W1*) was obtained. The water solubility index (*WSI*) and the *SP* were calculated as follows:

$$WSI = \frac{W1}{Weight\ of\ sample} * 100$$

$$SP = \frac{Ws}{(Weight\ of\ sample \times (100 - WSI))} * 100$$

Gel strength

Starch pastes (18%, w/v) in distilled water were heated and stirred in a water bath at 90°C for 30 min, and then cooled in an ice bath for 15 min. The paste was transferred into an aluminum can and sealed with paraffin wax to prevent moisture loss during storage. The gel samples obtained had dimensions of 25 mm diameter and 25 mm height. The starch paste was stored in a sealed plastic bag and kept at 4°C for 24 h and 48 h. The gel strength was measured with a Texture Analyzer (TA-XT2, Stable Micro Systems, Surrey, UK), using a TA cylinder probe (P/35). The parameters followed TA-XT2 settings: mode measure force in compression, pre-test speed: 0.2 cm/s, test speed: 10.0 mm/s, post-test-speed: 0.2 cm/s, distance: 2 cm, trigger type: auto-5 g, data acquisition rate: 200 pps. The peak force measured was reported as gel hardness, and the measurement was replicated three times for each sample.

Pasting temperature

The pasting properties of the starches were measured using a Rapid Visco Analyzer (Newport Scientific Instruments, Sidney, Australia). Starch dispersion in water (6%, w/w) was balanced, and held at 50°C for 1 min, then heated to 95°C at a rate of 6°C/min, then held at 95°C for 5.5 min and cooled from 95°C to 50°C at a rate of 6°C/min. Finally, the suspension paste was held at 50°C for 2 min. The paste was stirred at 160 rpm throughout the experiment.

RSM experimental design and statistical analysis

The effects of incubation and freezing times on resistant starch (RS) formation were investigated. Response surface methodology (RSM) was employed to optimize the preparation conditions of glass noodles by central composite design (CCD) of two factors, namely cold incubation time at 4°C from 0 to 9 h 30 min and freezing times at -13°C from 19 to 53 h. CCD was adopted to evaluate the combined effects of dependent variables with the two independent variables (storage time at 4°C and freezing time at -13°C). Five levels of each variable were selected to cover the process conditions (Table 1), and the axial distance was ±1.41. The complete design consisted of 13 treatments including 4 axial points, 5 vertices and the center point using Design-Expert® v.7.00 (Stat-Ease, Minneapolis, MN, USA). Three replications were carried out for all designs.

Table 1: Independent variable values of the storage and freezing times for prepared vermicelli and their corresponding levels

Variable	Symbol		Level				
	Coded	Unit	–1.41	–1	0	1	1.41
Storage times	A	h	0.76	2.00	5.00	8.00	9.24
Freezing times	B	h	19.03	24.00	36.00	48.00	52.97

To obtain vermicelli preparation, incubation times (4°C) were 0, 2, 5, 8, and 9 h 30 min and the freezing times (-13°C) were 19, 24, 36, 48, and 53 h, depending on the levels defined in the experimental design. An individual experiment was carried out for the regression model and fitted into an empirical quadratic polynomial model. Models were developed to describe the response (Y) in the form:

$$Y = \beta_o + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1\ j\neq 1}^{2} \beta_{ij} X_i X_J$$

where Y is the dependent or response variable and βo, βi, βii, and βij are the constant, linear, quadratic, and cross product regression coefficients, respectively. Xi are the coded independent variables of X_1 and X_2. The results were statistically tested by ANOVA at the significance level of $P = 0.05$. The adequacy of the model was evaluated by the coefficient of determination (R^2) and model P-value.

Vermicelli preparation

The procedure for vermicelli making followed Tan et al. (2006), and served as a reference method with a slight modification. One kilogram of raw starch mixture was made from 950 g of raw starch and 50 g of cooked starch. Cooked starch (pre-gelatinized starch) was prepared by mixing 50 g of raw starch with 250 mL of water and heating for 5 min. Mung bean starch and mung bean mixed cowpea starch in the ratio of 1:1 were added to water to obtain starch slurry. The dough moisture content was then adjusted to 50% by weight, and extruded using a hand operated mincer, pouring into a stainless steel cylinder 0.5 cm diameter directly into boiling water at 98 – 100°C. The dough was pressed with a steady rhythm flow line until cooked completely for 3 min, before transfer into cold water. The vermicelli floated on the surface of the water. After rinsing in cold water, the strands were drained and the vermicelli was refrigerated at 4°C of 0 to 9 h 30 min (A), and then frozen at -13°C of 19 to 53 h (B) (Table 1). The vermicelli was then dried at 45°C in an oven dryer of 8 to 12 h to obtain a final moisture content of 10 to 14%, and kept in polyethylene bags at room temperature.

Determination of the cooking quality

Cooking time, cooking yield, and cooking loss were determined following the method of 60 – 55 AACC (2000). The cooking yield of the cooked glass noodle samples was determined for 10 g (dry weight) boiled in 300 mL water, then washed with 50 mL of distilled water, drained for 5 min, and weighed immediately to calculate the percentage increase in weight using the following equation:

$$Cooking\ yield\left(\%\right)=\frac{M1-M2*(1-W)}{M2*(1-W)}*100$$

Where *M1* was the weight of the vermicelli after cooking (g), *M2* was the weight of the vermicelli before cooking (g), and *W* was the moisture content of the vermicelli before cooking (%).

To determine the cooking loss, the cooking water was collected, transferred to a moisture can, and dried at 105°C to constant weight. The cooking loss of the vermicelli was calculated using the following equation:

$$Cooking\ loss\left(\%\right)=\frac{M}{G*\left(1-W\right)}*100$$

Where *M* was the weight of the dried vermicelli (dry matter) (g), *G* was the weight of the vermicelli before cooking, and *W* was the moisture content of the vermicelli before cooking (%).

Texture analysis

The vermicelli (10 g) were cooked in water (120 mL) for 10 min, and then cooled to room temperature within 5 min. The vermicelli (25 – 30 cm) was placed in a straight line on a plate. Tensile strength was measured using a texture analyzer (TA-XT2, Stable Micro Systems, Surrey, UK), adapted from the method of Ritthiruangdej et al. (2011). Instrument settings for each treatment of 10 specimens included the extension mode, trigger type, auto 5.0 g, pre-test speed 3.0 mm/s, post-test speed 5.0 mm/s, test speed 3.0 mm/s, and trigger distance 100 mm. From the force-distance curves, two parameters of the vermicelli texture were obtained:tensile strength (maximum force; g), and breaking length (distance at maximum force; mm).

Statistical analysis

The experimental data were analyzed using analysis of variance (ANOVA), and expressed as mean value and standard deviation. Duncan's multiple range test was conducted to assess significant differences among the experimental mean values ($P < 0.05$). All statistical computations and analyses were conducted using SPSS version 13.0 for Windows. The Design Expert Software (version 7.0.0, Stat-Ease, Inc., Minneapolis, MN) was used for the statistical design of experiments and data analysis. ANOVA was used for analyses of the data to obtain the interaction between the process variables and the responses. The quality of the fitting model was expressed by the coefficient of determination R^2. Model terms were selected or rejected based on the *P*-value at 95% confidence level.

RESULTS AND DISCUSSION

Physical properties of starches

Solubility of starch

The solubility indices of mung bean, cowpea, and mixed starch were determined at 55, 65, 75, 85, and 95°C (Fig. 1 left). The mung bean starch had the highest solubility index at 1.09, 1.17, 1.77, 2.15 and 4.22%, respectively. Similar patterns of solubility for legume starch were observed by Liu and Shen (2007; Huang et al. (2007); and Li et al. (2011). However, the solubility of some starch mixtures increased rapidly at 85 and 95°C above the pasting temperature. The solubility of cowpea starch at 85°C reduced from 1.48 to 1.43% at 95°C. It is possible that the starch granules swelled when heated, blocking the water inside the structure. These results agreed with Shung and Stone (2004), who reported that the solubility of starch decreased as the temperature increased from 60 to 70, 80, and 90°C, with solubility index values of 0.3, 0.7, 5.0, and 1.1%, respectively. This may be because the gelatinized starch prevented the leaching of soluble material into the water. The water solubility of starch during cooking related closely to the texture and cooking quality of the vermicelli.

Swelling power of starch

The swelling power index (SWI) is the volume or weight of starch increase when the starch granules freely swell in water. The SWI at 55°C and 65°C was 2.22 to 2.36%, and 2.08 to 2.54%, respectively (Fig. 1 right). SWI fluctuated in a narrow range ($P < 0.05$), and in agreement with the results reported by Hoover and Sosulski (1985). When mung bean starch was mixed with cowpea starch the SWI increased as the cowpea starch added increased (Huang et al., 2007). The SWI of the starch mixture was low at 55 and 65°C. This may be because these temperatures are lower than the gelatinization temperature. The pasting temperature (Table 2) ranged from 74.58 to 77.30°C, similar to results reported by Gujiska et al. (1994), which indicated that legume starch had a two-stage swelling pattern. The crystal structure and amorphous bonding area in the starches remained. When the temperature increased to 75, 85, and 95°C, the hydrogen bonds holding the molecules

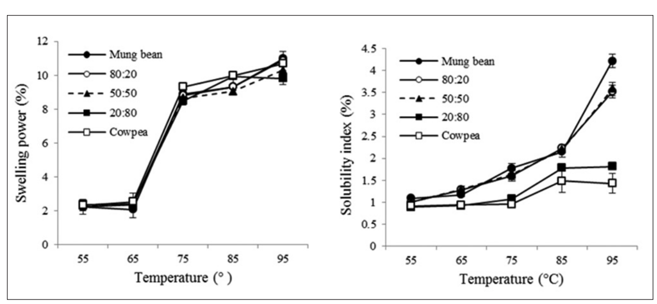

Fig 1. Swelling power and solubility index of mung bean (M) and cowpea (C) starch for different contents.

Table 2: Pasting properties of mixed mung bean (M) and cowpea (C) starches

M:C content	Pasting temp (°C)	Pasting properties (RVU)				
		Peak viscosity	Trough	Breakdown	Final viscosity	Setback
Mung bean	74.58±0.43[c]	621.21±2.10[a]	339.54±2.98[a]	280.11±3.00[a]	451.29±5.30[a]	107.61±7.69[a]
80:20	75.54±0.48[b]	603.90±3.58[b]	338.14±3.40[a]	265.62±3.14[b]	442.23±4.80[a]	104.73±4.38[ab]
50:50	75.36±0.07[b]	604.46±0.24[b]	338.35±2.50[a]	265.57±0.55[b]	441.23±1.09[a]	104.24±0.75[ab]
20:80	76.90±0.31[a]	564.66±2.40[c]	333.31±3.44[b]	226.75±1.45[c]	443.77±2.24[a]	101.16±1.39[bc]
Cowpea	77.30±0.45[a]	555.87±2.93[d]	334.67±1.47[b]	221.05±1.60[d]	443.04±5.92[a]	97.71±1.18[c]

Results are mean±standard deviation of triplicate determinations. Mean values of different letters a, b, c in the same column are significantly different (P<0.05)

of starch together weakened and were destroyed, allowing water molecules to diffuse faster into the starch granules, and the swelling power increased (Lee and Osman, 1991). A temperature of 75°C improved the SWI of the starch mixture. The SWI increased rapidly from 65 to 75°C in all samples. At 75°C the SWI varied from 8.48% (mung bean starch) to 9.32% (cowpea starch). When the temperature increased to 85°C and 95°C the SWI also increased. These results were consistent with the study by Sung and Stone (2004). They reported that the SWI of mung bean starch increased with an increase in temperature from 60, 70, 80, and 90°C, with values of 2.2, 2.8, 6.8, and 10.4%, respectively.

Gel strength of starch

The gel strength of the starch solution (18%, w/w), stored at 4°C for 24 h and 48 h increased significantly ($P < 0.05$), as the level of cowpea starch increased (Fig. 2).The strength of the starch gel incubated for 24 h ranged between 37.71 and 51.51 kgf. The highest value was shown by the cowpea starch, while the mung bean starch was lowest. However, the gel strength of the starch mixtures M:C (80:20), M:C (50:50), and M:C (20:80) were not significant, and ranged from 46.23 to 47.48 kgf ($P > 0.05$) after 48 h storage. These results were similar to Hoover et al. (1997) who found that the hardness of green bean and cowpea starch increased with storage time. This may be due to the higher retrogradation of gels implicated with hardness increase (Lee et al., 2005). The amylose content played an important role in starch pasting and gel strength as its high retrogradation, a desired property for noodle products (Tan et al., 2009).

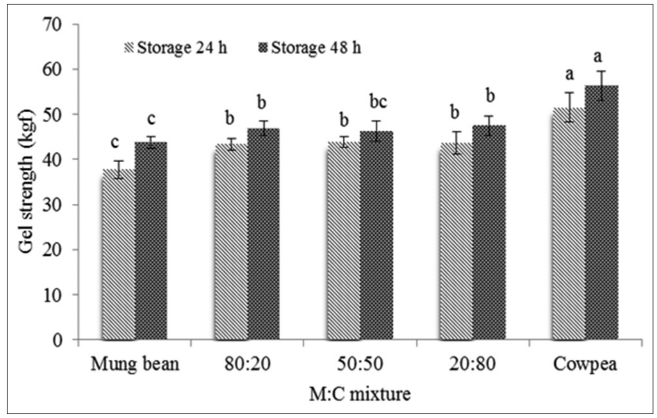

Fig 2. Gel strength of mung bean (M) and cowpea (C) starch at different ratios. Results are mean±standard deviation of triplicate determinations. Mean values of different letters a, b, c in the same bar graphs are significantly different ($P < 0.05$).

Pasting properties of starch

The pasting properties of the mixed starches were determined using a rapid visco analyzer (RVA) (Table 2). Results indicated that the mixture of mung bean and cowpea starch significantly affected the pasting properties ($P < 0.05$). The pasting temperature of the starch mixtures varied between 74.58 and 77.30°C, with mung bean starch the lowest and cowpea starch. Mung bean starch swelled rapidly at a lower temperature than cowpea starch. These pasting temperature results were similar to those of Thao and Noomhorm (2011; Li et al. (2011); and Park et al. (2012) who reported a range from 71.20 to 76.83°C. Huang et al. (2007) noted that cowpea starch had the higher pasting temperature (80°C). The peak viscosity represents the swelling of the starch granules during heating. This reflects the capability of the amylose molecules to hold the water that diffuses out of the starch granules. When the temperature was increased to 95 °C, the starch granules had maximum swelling and the molecules bonded (Greenwood, 1997). The mung bean starch showed the highest peak viscosity of 621.21 rapid viscosity units (RVU), and cowpea starch had the lowest at 555.87 RVU. For the mixed starches, the peak viscosity ranged between 564.66 and 604.46 RVU. When the cowpea starch ratio increased, the peak viscosity also decreased. These results were similar to those reported by Thao and Noomhorm (2011) and Park et al. (2012), who found that the highest viscosity of mung bean starch. The breakdown viscosity of mung bean starch was highest (280.11 RVU), with cowpea starch the lowest (221.05 RVU), while the breakdown values of the mixed starches ranged from 226.75 to 265.57 RVU. This trend was similar to the results of Park et al. (2012) who showed a breakdown range from 178.9 – 232.1 RVU. Increasing the cowpea ratio reduced the breakdown viscosity. The final viscosity of the mixed starches was not significantly different. The values ranged from 441.23 to 443.77 RVU, the mung bean starch was highest and the cowpea starch was lowest. The setback viscosity of the starch mixtures showed that cowpea starch had the lowest value (97.71 RVU) and mung bean starch the highest (107.61 RVU).

Therefore, from the physical properties of starches, the starch mixture of M:C 50:50 was selected to prepare vermicelli, and to apply RSM to optimize the most suitable process to obtain higher RS content. This starch mixture was used because its swelling properties, gel strength, and pasting properties were close to those of mung bean generally used in vermicelli production.

Response surface methodology (RSM) analysis for preparing vermicelli

Effect of processing variables on resistant starch content in uncooked and cooked vermicelli

The combined effect of the two dependent variables on the resistant starch (RS) of vermicelli is presented in Table 3.

The independent and dependent variables in Table 3 were regression and ANOVA analysis fitting the model and examine the statistical significance of the model (terms for estimated regression coefficients of quadratic polynomial models.) Their statistical significances were analyzed at a probability of 0.001, 0.01, and 0.05. The coefficients of variables in the equations were developed are presented in Table 4 and the statistical parameters were summarized as the determination coefficient (R^2), and the coefficient of variation (CV). ANOVA results showed a statistically significant relationship between the variables (the models) within a 95% confidence interval. Analysis of variance showed the model was highly possibility, the both of which could be used for measuring the correlation and significance of the models as shown Table 4. The lack of fit of both dependent variables was not significant. The coefficient of determination (R^2) values showed that the RS content of both uncooked and cooked vermicelli was high at 0.95 and 0.94, respectively. This indicated that a high proportion of variability was explained by the data. The RSM model was adequate and the coefficient of variation (CV) of cooked uncooked vermicelli and uncooked vermicelli were 3.53 and 5.83%, respectively, indicating good precision and reliability of the experiment.

Table 3: The central composite design (CCD) showing the effect of the independent variables on the two dependent variables

Run	Independent variables[1]				Dependent variables	
	Coded levels		Time (true values)		RS content (Y) (%)	
	X_1	X_2	A^2 (h)	B^3 (h)	Uncooked[4]	Cooked[5]
1	1.41	0.00	9 h 30 min	36.00	22.31	14.25
2	−1.00	1.00	2.00	48.00	20.14	10.65
3	0.00	0.00	5.00	36.00	20.14	11.45
4	0.00	−1.41	5.00	19.00	27.25	17.25
5	1.00	−1.00	8.00	24.00	24.12	14.25
6	1.00	1.00	8.00	48.00	22.15	13.25
7	0.00	0.00	5.00	36.00	20.25	10.58
8	0.00	1.41	5.00	53.00	20.32	11.58
9	0.00	0.00	5.00	36.00	19.25	10.58
10	−1.00	−1.00	2.00	24.00	26.12	16.58
11	0.00	0.00	5.00	36.00	19.54	10.38
12	−1.41	0.00	0.00	36.00	20.25	11.75
13	0.00	0.00	5.00	36.00	20.58	11.87

[1]There was statistically significant difference between groups. [2]A and X_1, storage times at 4°C (h). [3]B and X_2, freezing times at −13°C (h). [4]Y_1 represents the RS yield (%) of uncooked vermicelli. [5]Y_2 represents the RS yield (%) of cooked vermicelli

Effect of incubation and freezing times on the RS content of uncooked vermicelli

The maximum RS content of uncooked vermicelli observed in the model was 27.25%, while the minimum was 19.25% (Table 3). Analysis of the regression coefficient indicated that the freezing time had strong linear ($P = 0.0002$) and quadratic effects on the RS content of uncooked vermicelli. However, analysis of the regression coefficient for the incubation times indicated that storage time had no significant effect on the RS content of uncooked vermicelli ($P > 0.05$). The response surface for the RS content of uncooked vermicelli showed areas as presented in Fig. 3. The freezing time was most effect on RS content, and the response surface of uncooked vermicelli showed highest RS content with freezing times between 19 and 24 h. The longer freezing times (30 to 52 h) did not affect the RS content. The quadratic level of storage and freezing times had a positive effect on the RS content ($P < 0.05$), and the RS content increased as these variables increased. Interactive storage and freezing times showed that the RS content was high at incubation times between 1 and 3 h 30 min, and freezing times between 19 and 24 h.

The regression equation described the effect of the process variables (incubation and freezing times) on the resistant starch content of uncooked (Y_1) and cooked (Y_2) vermicelli. New prediction models, expressed in terms of coded variables for each dependent variable were developed (Table 4). The maximum RS of cooked vermicelli in the model was 17.25%, while the minimum was 10.38% (Table 3). Analysis of the regression coefficient (Table 4)

Table 4: ANOVA of regression for the RS contents of uncooked and cooked vermicelli

Model	Lack of fit	R^2	Equation[1]	Significant model[3]
Quadratic significant	Not significant	0.95	$Y_1 = 20.01+0.45X_1-2.22X_2+1.00X_1X_2+0.65X_1^2+2.08X_2^2$	B***, AB*, A[2]*, B[2]***
Quadratic significant	Not significant	0.94	$Y_2 = 11.02+0.58X_1-1.87X_2+1.23X_1X_2+0.84X_1^2+1.74X_2^2$	B***, AB*, A[2]*, B[2]***

[1]Y_1 and Y_2 represent RS contents of uncooked and cooked vermicelli (%), respectively. [2]A and X_1, storage times at 4°C (h) and B and X_2, freezing times at −13°C (h). [3]*Significant at P=0.05 level, **Significant at P=0.01 level, ***Significant at P=0.001 level

indicated that the freezing time had both a linear and quadratic effect on the RS content of cooked vermicelli ($P = 0.0004$). In the responses areas (Fig. 4), the freezing time affected RS content. For cooked vermicelli, the RS content was highest for freezing times between 19 and 22 h. longer freezing times (25 to 52 h), the RS content decreased to values similar to uncooked vermicelli. The incubation times also showed no significant effect on the RS content of cooked vermicelli ($P > 0.05$). The interactive terms of incubation and freezing times (quadratic level) had a positive effect on the RS content ($P < 0.05$), and the RS content increased with increase of these variables. The maximum RS content was given for storage time between 1 h 30 min and 4 h, and freezing time between 19 and 22 h. The RS content decreased approximately 10.38 to 17.25% from the initial level when boiled at 98–100°C for10 min.

The RS content was improved because during cooling and freezing starch regains an ordered structure. This is called retrogradation, and makes the starch more resistant to digestion (Cui and Oates, 1997; Chung et al., 2006). In vermicelli preparation, a high degree of syneresis and accelerated retrogradation (RS3) was formed during the cooking and cooling periods, and the amylose and amylopectin in the starch molecules were recrystallized (Zhang et al., 1999). The increase of RS was also supported by Garcia-Alonso et al. (1999) who found that freezing conditions increased RS levels. At constant temperature, the linear chains of amylose molecules re-crystallize (double-helical crystallites) faster, producing the initial decreased digestibility of retrograded starch. On the other hand, the re-crystallization of amylopectin molecules over long time periods of several days form crystallites because of the short branch chains and branching structure of amylopectin (Chung et al., 2006; Zhou and Lim 2012). Therefore, high RS glass noodles could be obtained by maintaining a low temperature, as supported by the study of Galvez et al. (1994). Moreover, Lilijeberg et al. (1992) found that steamed and roasted potatoes stored at 4 °C for 24 h contained 31.05 and 52.50% RS content, respectively. This was higher than fried potato at 5.19%, which probably contained retrograded amylose.

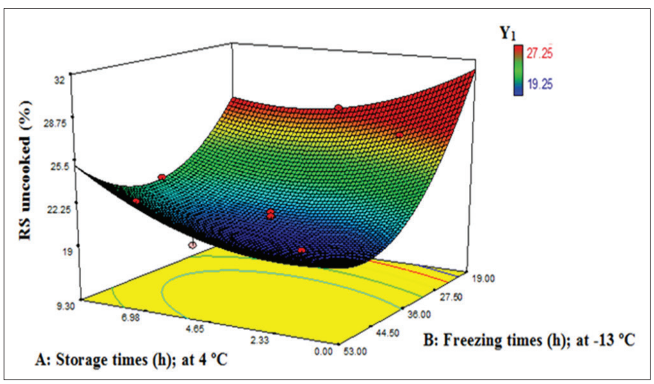

Fig 3. Response surface for the effect of storage and freezing times on the resistant starch (RS) content of uncooked vermicelli.

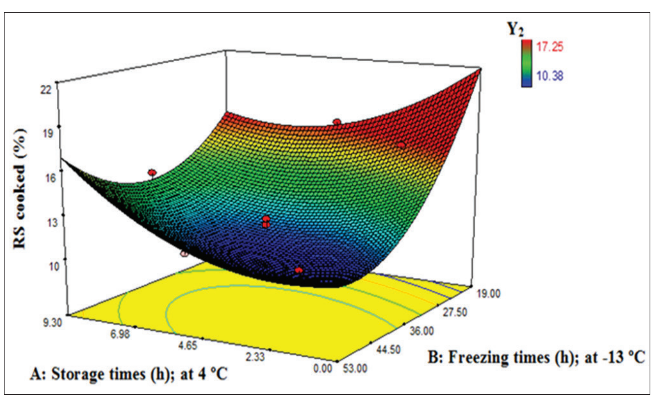

Fig 4. Response surface for the effect of storage and freezing times on the resistant starch (RS) content of cooked vermicelli.

The optimal conditions for the preparation of high RS in vermicelli

The optimal process conditions for preparing high RS vermicelli were achieved using design expert software. In this study, the independent variables were considered optimum if the dependent variables (RS of uncooked Y_1 and cooked Y_2 vermicelli) were as high as possible. The best conditions were found to be storage at 4 °C for 1 h 21 min, and freezing at -13°C for 20 h 54 min. The models gave predicted values of Y_1 and Y_2 as be 28.36 and 18.87%, respectively.

For the experiments carried out to confirm the adequacy of the models in predicting the values of the dependent variables (Y_1 uncooked, and Y_2 cooked vermicelli), the incubation of the noodles at 4°C for 1 h 30 min and frozen at -13 °C for 21 h were applied. The results are indicated in Table 5 showing the optimum conditions of starch mixture M:C (50:50), compared with the vermicelli prepared from starch mixture M:C (50:50) using reference method (Tan et al., 2006) on the cooking quality and texture properties.

The texture, cooking qualities, and RS contents of vermicelli prepared using optimum conditions

The texture of the vermicelli was measured by the tensile strength and the elasticity (Table 5). The tensile strength values of the vermicelli ranged between 0.03–0.04 kgf. The harder texture noodle could be related to negative rehydration and tensile stress. Thao and Noomhorm (2011), reported that the tensile strength of mung bean noodles was 5.24 kgf/mm^2. This result was similar to Kasemsuwan et al. (1998) who prepared mung bean noodles with different ratios of dry and gelatinized starch at (1:1), (2:3), and (1:2). The tensile strengths were 5.51, 4.95, and 2.49 kgf/mm^2, respectively. The elasticity values of the vermicelli are shown in Table 5.The results show

Table 5: Texture and cooking qualities for optimum condition compared with the control vermicelli

M:C content	A[1](h)	B[2](h)	Tensile (kgf)	Elasticity (mm)	Time (min)	Loss (%)	Yield (%)
50:50	1 h 30 min	21	0.03±0.002[ab]	28.58±0.51[b]	10.50±0.04[b]	3.43±0.24[c]	349.41±3.69[a]
50:50[3]	2	24	0.03±0.001[b]	27.58±1.05[b]	10.82±0.53[ab]	3.65±0.10[c]	323.89±1.45[b]
Mung bean[3]	2	24	0.04±0.001[a]	34.69±1.31[a]	10.33±0.09[b]	6.06±0.01[a]	303.96±2.57[c]
Cowpea[3]	2	24	0.03±0.001[b]	26.84±1.06[b]	10.24±0.18[a]	4.33±0.22[b]	289.00±3.15[c]

Mean values of different letters a, b, c in the same column are significantly different (P<0.05). [1]A, Incubation times at 4°C. [2]B, Freezing times at −13°C. [3]Noodles obtained from the reference method (incubated at 4°C for 2 h and freezing at −13°C for 24 h)

that the elasticity varied from 26.84–34.69 mm. The highest elasticity (34.69 mm) was found in mung bean starch (control). The vermicelli made from M:C (50:50) at optimum condition (storage at 4 °C for 1.3 h and freezing at -13°C for 21.00 h) had elasticity of 28.58 mm, not significant with M:C (50:50) of control (27.58 mm). However, the elasticity of vermicelli made from cowpea starch was lowest at 26.84 mm, possibly due to its high solubility and swelling power. Mung bean starch contains high amylose which can form a firm and highly elastic gel (Biliaderis et al., 1985).

Cooking qualities are important factors for vermicelli products. The cooking time, cooking loss, and cooking yield of vermicelli are shown in Table 5. The shortest cooking time was found for the cowpea vermicelli (10.24 min). This may be due to the difference in gelatinization temperature. Starch high in gelatinization resulted in longer cooking times with lower cooking loss (Waniska et al., 1999). The cooking loss indicates the ability of the vermicelli to maintain their structure. The vermicelli had cooking losses ranging from 3.43% –6.06%. The cooking loss of mung bean vermicelli was highest at 6.06%. However, the vermicelli made from M:C (50:50) of optimum condition and control were not significantly different from cowpea starch. Cooking loss is detrimental to noodle products. If the vermicelli dissolves in the water, the broth becomes cloudy and the noodles are sticky in the mouth (Jin et al., 1994). A low cooking loss for noodle products indicates good noodle quality. Consumers prefer cooking losses lower than 10% (Tan et al., 2009). Shung and Stone (2004) reported that the cooking loss of starch noodles was positively related (P < 0.05 and R= 0.90) to the solubility of the legume starch, but negatively correlated with the swelling power (R= -0.56). The cooking yields of the vermicelli differed significantly (P < 0.05) (Table 5). The vermicelli prepared from the M:C (50:50) of optimum condition and control had high cooking yields of 349.41 and 323.89%, respectively. However the vermicelli prepared from mung bean or cowpea showed lower cooking yields than the mixed starch. The cooking yield relates to the swelling power and the internal structure of the starch. A larger swelling power resulted in higher cooking yield and rehydration (Kim et al., 1996). This affected the swelling, which is desirable for the manufacture of noodle products.

Table 6: The real experimental results of RS contents of optimum condition compared with the control vermicelli

M:C content	Real values		RS content (%) of vermicelli	
	A[1](h)	B[2](h)	Uncooked	Cooked
50:50	1h 30 min	21	29.20±0.81[a]	19.41±0.24[a]
50:50	0	36	20.45±0.78[c]	11.25±1.21[c]
50:50	5	53	20.32±0.95[c]	11.86±0.54[c]
50:50[3]	Control		23.93±0.53[b]	13.86±0.31[b]
Mung bean[3]	Control		23.83±0.19[b]	13.23±0.58[b]
Cowpea[3]	Control		23.37±0.73[b]	13.47±0.77[b]

Mean values of different letters a, b, c in the same column are significantly different (P<0.05). [1]A, Storage times at 4°C and [2]B, Freezing times at −13°C. [3]Control noodles: storage at 4°C for 2 h and freezing at −13°C for 24 h

The high cooking yield of vermicelli results from water absorption which makes the noodles softer and more cohesive.

The RS content of vermicelli obtained using the optimum conditions compared with the reference vermicelli

The RS contents were measured for both uncooked and cooked vermicelli. The results are presented in Table 6. The RS contents of dried vermicelli ranged from 20.32 to 29.20%. The noodles made from M:C (50:50) contained the highest RS content, followed by the vermicelli prepared by the reference method (control). The appropriate time to obtain the maximum RS formation was freezing at -13°C of 21 to 24 h. These conditions accelerated the retrogradation of amylose molecules, in agreement with Cui and Oates (1997) and Chung et al. (2006). Moreover, the results in Table 6 show that the RS content of vermicelli frozen at -13°C for longer times of 36 to 53 h, did not increase. This may be because with longer storage the rate of amylopectin retrogradation was slow and several days are required to form crystallites (Cui and Oates, 1997; Chung et al., 2006).Cooking vermicelli at 98 °C to 100°C for 10 min caused significant loss (approximately 10%) of RS. The remaining resistant starch was highest (19.41%) in the cooked vermicelli M:C (50:50) with optimum conditions. These changes may be due to the structural modification of components during storage. Niba (2003) found that the storage time and temperature of whole corn bread and corn bread crumbs affected RS formation. The RS content reached a maximum for storage between 2 and 4 days at -20°C. RS content decreased with longer storage (after 7 days). Therefore, the experiment confirmed the closeness of model fit, and the experimental results for optimum conditions to prepare high RS in vermicelli. As a result, the vermicelli has applications as a resistant starch food to improve human health.

CONCLUSIONS

The starch mixture M:C (50:50) was selected to prepare vermicelli and used in the optimization process as its physical properties were close to those of mung bean starch. The best conditions for preparing high RS content (29.20%) were incubation at 4°C for 1h 30 min, followed by freezing at -13°C for 21 h. The modeling of experimental data allowed the generation of useful equations for general use in predicting the quality of vermicelli under different combinations of factors.

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Author contributions

R. Photinam participated in the experiments and data analysis, and also contributed to the writing of the manuscript. A. Moongngarm, the corresponding author designed the research plan, organized the study, participated in experiments, coordinated the data analysis, and contributed to the writing of the manuscript. T. Paseephol participated in the experimental design and coordinated the data analysis.

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