บทความวิจัย

ผลของการเติมแป้งข้าวสีม่วงต่อความคงตัวและปริมาณฟีนอลิก ในโยเกิร์ตปราศจากไขมันชนิดคงตัว

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเติมแป้งข้าวสีม่วง (1.0, 3.0 และ 5.0% w/w) ในโยเกิร์ตปราศจากไขมันชนิดคงตัว ในสภาวะที่เติมแป้งข้าวโพด (0.75% w/w) และไม่เติมแป้งข้าวโพด ต่อความคงตัวและปริมาณฟีนอลิกของโยเกิร์ต จากผลการศึกษาพบว่าโยเกิร์ตที่มีการเติมแป้งข้าวสีม่วง (5.0%) ร่วมกับแป้งข้าวโพด (0.75%) มี % การแยกตัวของเวย์และ % syneresis เท่ากับ 0.00% และ 2.19% ตามลำดับ โยเกิร์ตที่มีการเติมแป้งข้าวสีม่วงอย่างเดียว (5.0%) มีค่าการแยกตัวของเวย์และ % syneresis เท่ากับ 0.86% และ 6.48% ในขณะทีโยเกิร์ตควบคุม (ไม่เติมแป้งทั้ง 2 ชนิด) มีค่าเท่ากับ 3.47% และ 33.87% ซึ่งมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) โยเกิร์ตที่มีการเติมแป้งข้าวสีม่วง (5.0%) ร่วมกับแป้งข้าวโพด (0.75%) มีความแน่นเนื้อสูงที่สุด โดยมีค่าเท่ากับ 30.87 g และโยเกิร์ตที่มีการเติมแป้งข้าวสีม่วง (5.0%) เพียงอย่างเดียว มีค่าความแน่นเนื้อเท่ากับ 26.50 g ส่วนโยเกิร์ตควบคุมมีค่าความแน่นเนื้อเท่ากับ 21.92 g และเมื่อทำการวิเคราะห์ปริมาณฟีนอลิกพบว่าปริมาณฟีนอลิกเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($p < 0.05$) เมื่อความเข้มข้นของปริมาณแป้งข้าวสีม่วงเพิ่มขึ้น

คำสำคัญ: โยเกิร์ตชนิดคงตัว แป้งข้าวสีม่วง ปริมาณฟีนอลิก เนื้อสัมผัส

สาขาวิทยาศาสตร์การอาหารและโภชนาการ คณะเทคโนโลยีและนวัตกรรมผลิตภัณฑ์การเกษตร มหาวิทยาลัยศรีนครินทรวิโรฒ
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Effect of Purple Rice Flour Addition on Stability and Total Phenolic Contents of Non-Fat Set Yogurt

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ABSTRACT

This study aims at exploring the effect of addition of purple rice flour (PRF) at 1.0, 3.0 and 5.0% (w/w) to fat free yogurts with or without corn starch (CS) at 0.75% (w/w) on stability and total phenolic contents of yogurts. Yogurt made with addition of 5.0% PRF and 0.75% CS had % spontaneous whey separation and % syneresis of 0.00% and 2.19%, respectively. Addition of 5.0% PRF without CS showed spontaneous whey separation and syneresis values of 0.86% and 6.48%, respectively, compared to significantly lower values of 3.47% and 33.87% found in the control yogurt ($p < 0.05$). Firmness of yogurt made with addition of 5% PRF combined with 0.75% CS and 5% PRF (without CS) was at 30.87 g and 26.50 g, respectively, while the control yogurt had the lower firmness value of 21.92 g ($p < 0.05$). Total phenolic contents significantly increased with an increase in concentration of added PRF ($p < 0.05$).

Keywords: Set-type yogurt, Purple rice flour, Phenolic contents, Texture

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Introduction

Yogurt is a fermented dairy product widely consumed throughout the world because of its nutritional values and health benefits, which primarily link to live microorganisms. Regular consumption of yogurt has been reported to improve immune system and lactose intolerance, and reduce constipation, diarrhea, risk of colon cancer, and inflammatory bowel disease [1]. The production of yogurt is typically carried out by fermentation of two strains of lactic acid bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* [2]. Gelation of milk is induced by acidification, which results in formation of protein network and texture of yogurt [3]. Whey separation, related to an unstable gel network and excessive rearrangement of the casein particles after gel formation, is a major defect of set-style yogurt [4]. Stabilizers, such as pectin, gelatin and starch, are generally added to prevent whey separation [5].

Besides nutritional values, yogurt also contains bioactive peptides, which possess antioxidant activity. Lactic acid bacteria are predominantly responsible for the production of these bioactive peptides from proteolytic activities of the microorganisms [6]. Food industry, however, attempt to improve quality of yogurt with high antioxidant potential [7]. Thus, addition of natural antioxidants to yogurt could be a potential functional food product.

Antioxidants are compounds that play a vital role in inhibiting oxidation reactions in foods and decrease adverse effects from free radicals such as reactive oxygen species that are constantly produced in human body [7, 8]. Natural antioxidants, found in many plant and herb extracts, have been used as a source of antioxidants in foods [9]. Recently, purple rice, 'Riceberry' type (*Oryza sativa* L.), has been broadly consumed because of many health-promoting compounds including antioxidants, such as γ-oryzanol, anthocyanin and phenolic compounds, and dietary fiber [10]. Thus, purple rice flour (PRF) can be potentially utilized as a natural source of antioxidants in yogurt. Apart from antioxidative potential, purple rice flour may help to improve yogurt stability and decrease whey separation. Addition of rice flour to yogurt, however, might lead to changes in yogurt physicochemical and textural properties because starch hydration and gelatinization in a mixed system of milk and starch may alter characteristics of final products [11].

This study aims at exploring the impact of addition of PRF to fat free yogurts with or without conventional yogurt stabilizer, corn starch (CS). Viscosity of yogurt premix samples prior to inoculation, pH profiles during fermentation, spontaneous whey separation, syneresis, firmness, total phenolic contents and color of non-fat set yogurts were determined.

Materials and Methods

1. Preparation of Yogurt Premix

Pasteurized skim milk acquired from a local supermarket was heated to 85°C for 30 min using a heating magnetic stirrer [12]. Heat treatment of milk was done to increase yogurt firmness by formation of disulfide bridging between denatured β-lactoglobulin and κ-casein on the casein micelle surface [5]. PRF, purchased from a local market, was milled using laboratory scale mill, and sieved through 100 mesh screen to obtain smaller and homogeneous particles. PRF (1.0, 3.0 and 5.0 w/w), CS (0.75%, w/w) or a combination of PRF (1.0, 3.0 and 5.0% w/w) and CS (0.75% w/w) were added to milk during heating. The premix samples were left overnight at 4°C before inoculation.

2. Apparent Viscosity of Premix Samples

To study the influence of added PRF on yogurt premix, apparent viscosity was determined at ~25°C using a Brookfield Viscometer Model DV-3T equipped with small sample adapter and SC4-31 spindle (MA, USA). A rotation speed of 40 rpm was applied. The measurement was done in triplicate for each sample.

3. Preparation of Culture

Yogurt culture YC-380 Yo-Flex (Chr.Hansen, Hørsholm, Denmark), a mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus,* was used as a starter culture. The stock culture was prepared by inoculating 130 mg of freeze-dried culture to 1000 g of autoclaved skim milk and incubating at 40°C overnight [12]. Stock cultures were then stored at -45°C. Working cultures were prepared by thawing frozen stock cultures, and 0.8 ml of thawed culture was inoculated to 80 g of autoclaved skim milk [12]. Milks were incubated at 40°C overnight before use.

4. Yogurt Fermentation

Premix samples (with addition of PRF, CS, or PRF+CS) were inoculated with 2% (w/w) inoculation rate of working culture and were incubated at 40°C until the pH level decreased to 4.6. Changes in pH levels during fermentation were monitored every 10 min using a pH meter Model Eutech pH 700 (Eutech Instrument, IL, USA). Yogurts were stored in a refrigerator at 4°C until analyses were done.

5. Spontaneous Whey Separation

The method developed by Lucey et al. [13] was used to determine spontaneous whey separation of the gels. After inoculation of working culture to premix samples, 220 g of inoculated samples were transferred to 250 ml glass volumetric flasks. Samples were incubated at 40°C until pH 4.6 was reached. Eight flasks were used for each trial. Whey appeared on the surface of yogurts after incubation was weighed. Percent whey separation was calculated using equation (1):

$$\%\ \text{Whey} = \frac{\text{Weight of whey (g)}}{\text{Total weight of milk (g)}} \times 100 \qquad (1)$$

6. Syneresis

Yogurt gels formed in a 50-ml conical plastic centrifuge tube were stored at 4°C for 16-20 h before analysis. Syneresis of yogurt was determined by the weight percentage of whey released after centrifugation of yogurts at 640 g at 4°C for 10 min using Sorvall Legend XTR Centrifuge (Thermo Scientific, MA, USA). Percent (%) syneresis is calculated as shown in equation (2) [14].

$$\%\ \text{Syneresis} = \frac{\text{Weight of whey (g)}}{\text{Total weight of milk (g)}} \times 100 \qquad (2)$$

7. Firmness

The analysis was carried out on the yogurts after 16-20 h of storage using a method described in Krasaekoopt et al. [15] with some modifications. Firmness of yogurts at 4°C was determined using a compression test carried out with a TA.XTplus Texture Analyser (Stable Micro Systems, Surrey, UK). A 20 mm acrylic cylinder probe was used. The test speed was fixed at 1 mm/s and the penetration depth was 10 mm. Firmness is expressed as gram (g), which is a peak force of compression. An average of 3 measurements was acquired from each sample, and 3 replications were applied for each treatment.

8. Total Phenolic Contents

Eight milliliters of ethanol were added to 2 ml of yogurt sample after 16-20 h of storage at 4°C, and centrifuged at 351 *g* for 30 min. The supernatant was retained for total phenolic content analyses. Total phenolic contents were determined using the Folin-Ciocalteu assay described by Singleton et al. [16] with some modifications. The yogurt extracts (0.5 ml) were mixed with distilled water (0.5 ml) and Folin-Ciocalteu reagent (0.5 ml). After mixing, sodium carbonate solution (2 ml, 20% w/v) was added, and the mixture was kept in the dark for 30 min at room temperature. Absorbance at 725 nm was read. The calibration curve was constructed using gallic acid, and the results were expressed as μg gallic acid equivalents (GAE) per gram of sample.

9. Color

Color of yogurt samples was expressed in L^*, a^*, b^* color space using a colorimeter, Color Flex EZ (Hunter Associates Laboratory, Inc., VA, USA). The test was done in triplicate.

10. Statistical Analysis

Analysis of variance was carried out using SigmaPlot 11.0 (Systat Software, CA, USA). The level of significant difference was determined at $p < 0.05$. The differences between means were analyzed using Duncan's multiple range test.

Results

1. Effect of PRF and CS on Apparent Viscosity of Premix Samples and Fermentation Time

To study the effect of addition of PRF, the apparent viscosity of yogurt premix samples was determined as shown in Table 1. Viscosity of samples significantly increased as the concentration of PRF increased. Addition of CS at 0.75% (w/w) without PRF significantly increased the viscosity of the premix sample. Combining CS and PRF further increased the viscosity of samples. An increase in viscosity of samples with added PRF, CS or a combination of PRF and CS resulted from a swelling and gelatinization process of starch granules when heat was applied at 85°C for 30 min. Pasting temperatures of PRF and CS were reported at ~ 80°C [17] and ~ 83°C [18], respectively, so the heat treatment applied in this study was sufficient for starch gelatinization to take place in milk. Starch granules in PRF and CS were able to absorb a large amount of water, thus the viscosity of the solution increased [19]. In addition, PRF also contains dietary fiber [20], which might help to increase the viscosity of the premix samples.

Table 1 Apparent viscosity of premix samples at 25°C determined at a rotation speed of 40 rpm, and fermentation time of yogurts at 40°C.

Sample	Viscosity (mPa.s)	Fermentation Time (min)
Control	3.75 ± 0.00^{f}	230 ± 10^{a}
1% PRF	5.25 ± 0.00^{e}	210 ± 20^{bc}
3% PRF	8.25 ± 0.00^{d}	203 ± 6^{bc}
5% PRF	14.25 ± 0.00^{b}	173 ± 6^{d}
0.75% CS	8.63 ± 1.59^{d}	213 ± 6^{b}
1% PRF+0.75% CS	11.63 ± 0.53^{c}	207 ± 6^{bc}
3% PRF+0.75% CS	12.75 ± 0.00^{c}	177 ± 6^{d}
5% PRF+0.75% CS	21.38 ± 0.53^{a}	193 ± 6^{c}

$^{a-f}$Different superscripts in the same column were significantly different ($p < 0.05$).

The pH profiles of yogurts are shown in Figure 1. The pH of all samples during fermentation decreased slowly for the first 70 min (from pH ~6.5 to ~6.0), and decreased rapidly until 150 min of fermentation, and thereafter the pH level decreased slowly when it was close to 4.6. These changes in pH profiles corresponded to lag phase (slow pH decline), logarithmic phase (rapid pH decrease) and slow acidification rate of starter bacteria [21].

Addition of PRF, CS and PRF+CS increased acidification rate, thus the time to attain pH 4.6 for yogurts with added PRF, CS and PRF+CS was significantly shorter than the control sample as shown in Table 1. Lactic acid is produced by thermophilic lactic acid bacteria during their growth. Elements in milk, such as, soluble phosphate and colloidal calcium phosphate contribute to the buffering capacity of milk [22]. Addition of PRF, CS and PRF+CS, therefore, might lower the buffering capacity of milk. Thus, it resulted in greater acidification rate. This result is in agreement with the study conducted by Zare et al. [23], who found that supplementation of 3% lentil flour to milk increased the acidification level of yogurt by lowering buffering capacity of milk when compared to control sample. Moreover, an increase in acidification rate was possibly due to prebiotic effect of dietary fiber containing in PRF [24].

Therefore, addition of PRF, CS or PRF+CS increased viscosity of premix samples as a consequence of gelatinization of added polysaccharides, and it reduced fermentation time by increasing acidification rate as a result of lower buffering capacity of milk when PRF, CS or PRF+CS was added.

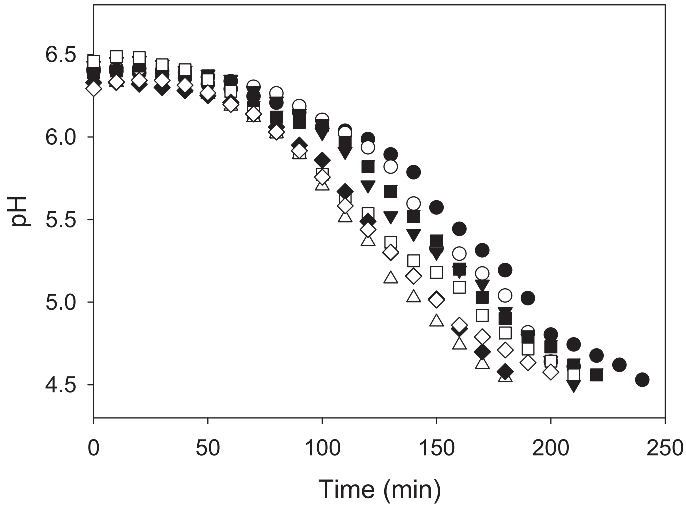

Figure 1 pH profiles of yogurts made with milk with addition of purple rice flour (PRF), corn starch (CS), or a combination of PRF and CS as a function of fermentation time: Control (●), 1% PRF (○), 3% PRF (▼), 5% PRF (△), 0.75% CS (■), 1% PRF+0.75% CS (□), 3% PRF+0.75% CS (◆), and 5% PRF+0.75% CS (◇). Incubation temperature was at 40°C.

2. Syneresis and Spontaneous whey separation

Syneresis and spontaneous whey separation were shown in Figure 2A and 2B, respectively. Syneresis of yogurt ranged from 2.19% to 33.87%, while whey separation ranged from 0% to 3.47%. Addition of PRF and CS caused a significant reduction in % syneresis and whey separation. Control sample (no PRF and CS added) exhibited highest % syneresis and whey separation. Addition of CS alone (no PRF) or PRF alone (no CS) significantly reduced syneresis and whey separation. Addition of PRF+CS reduced syneresis and whey separation to a greater extent compared to samples made with the same levels of PRF without addition of CS. Samples with addition of 5% PRF + 0.75% CS showed the lowest values for both characteristics.

Whey separation refers to the spontaneous appearance of whey on the surface of gels [25], and is related to instability of the gel network [25], while syneresis refers to the level of whey separated from the collapsed milk gels as affected by external force [26], and it measures water holding capacity of the gels under high forces [25]. In this study, however, similar trends were observed for both syneresis and whey separation. It might be explained as the presence of polysaccharides from both PRF and CS in the gels reduced whey separation and syneresis by retaining substantial quantities of serum in the gel structure [27] and increasing water binding capacity and viscosifying ability. PRF and CS used in this study were uncharged, thus they did not interact with milk proteins through electrostatic interaction. PRF and CS rather promoted an cosity of the liquid phase of the gels, hence it resulted in a decrease in phase separated domains [28]. This results are in agreement with Lobato-Calleros et al. [29] who reported lower % syneresis from yogurt made with addition of starch, and they concluded that added starch increased the water binding capacity of milk gels.

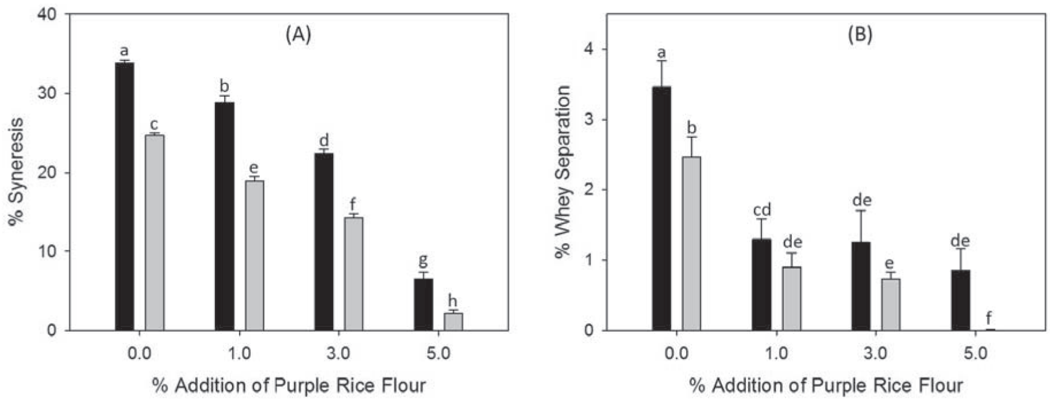

Figure 2 Syneresis (A) and Spontaneous whey separation (B) of yogurts made with addition of purple rice flour (PRF, ■), and a combination of purple rice flour and corn starch (PRF+CS, ■).[a-h] Different letters were significantly different ($p < 0.05$).

3. Firmness

Firmness is one of important attributes of yogurt. Structure of yogurt forms by aggregation of caseins by reduction of pH during fermentation and disulfide bonding between κ-caseins and denatured whey proteins when milk was heated to 85°C for 30 min during preparation of premix samples [25]. Addition of PRF and CS resulted in an increase in texture firmness as shown in Figure 3. The difference between firmness of yogurts made with and without addition of CS at the same level of PRF was not observed. PRF contains carbohydrate, protein, fiber and fat approximately at 76, 8, 3 and 1 g/100 g, respectively [20]. Addition of PRF to milk probably led to incompatibility between casein micelles and polysaccharides, the main component in PRF, and depletion flocculation could occur [30]. Depletion flocculation is described as the formation of a depleted layer around the casein micelles when mixed with other biopolymers, with difference in osmotic pressure, which drives the casein micelles to attract one another [28]. This phenomena might enhance the firmness of gels by formation of more protein-protein bonds. Similar results were observed by Tamime et al. [31], in which they found an increase in firmness of low fat yogurt when starch was added as fat substitute, but high concentration of starch impaired the flavor and mouthfeel of yogurts. Oh et al. [11] reported an increase in hardness of yogurt when potato starch was added and they concluded that the added starch absorbed water during heating, which resulted in an increase in the concentration of milk protein, so the density of the casein network increased.

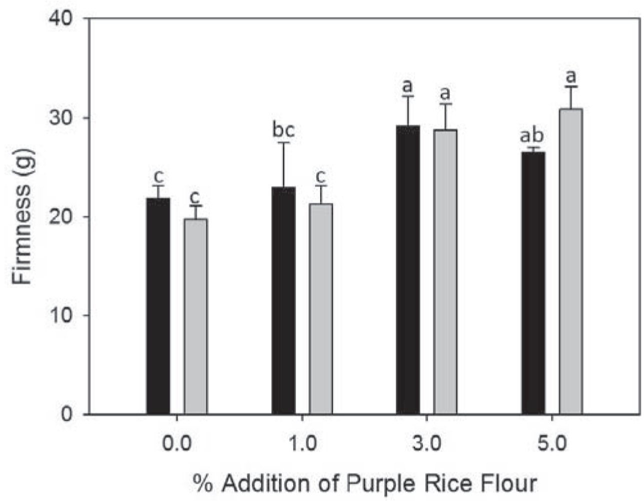

Figure 3 Firmness of yogurts made with addition of purple rice flour (PRF, ■), and combination of purple rice flour and corn starch (PRF+CS, ■).[a-c] Different letters were significantly different ($p < 0.05$).

4. Total Phenolic Contents

Total phenolic contents are shown in Figure 4. Total phenolic contents of yogurts significantly increased with concentration of added PRF. These results were in agreement with a study by Muniandy et al. [7], in which tea extracts was added to yogurts, and it resulted in elevated total phenolic contents of samples. Besides total phenolic contents, the antioxidant capacity of yogurts fortified with tea extracts was also increased [7]. Therefore, it is likely that yogurts made with addition of PRF have increased antioxidant capacity.

However, without addition of PRF, the total phenolic contents of yogurt with addition of 0.75% CS was lower than the control. This reduction in total phenolic contents is not yet fully understood, but this behavior was possibly due to an increase in ability of the gel to retain serum phase and increased viscosity of samples when CS was added, thus the extractable phenolic compounds during centrifugation became smaller.

Heat treatment (85°C 30 min) applied in this study might influence the total phenolic contents of samples. Zaupa et al., [32] found that hydrothermal treatment (boiling) of pigmented rice resulted in a reduction in total phenolic contents and antioxidant capacity of all pigmented rice varieties (white, red and black rice).

Phenolic compounds and anthocyanins found in pigmented rice have been recognized as health-promoting substances as they possess antioxidant, anti-inflammatory and anticancer properties [33]. In addition, Chatthongpisut et al. [10] found that extract from Thai purple rice had antiproliferative effect against human colon cancer.

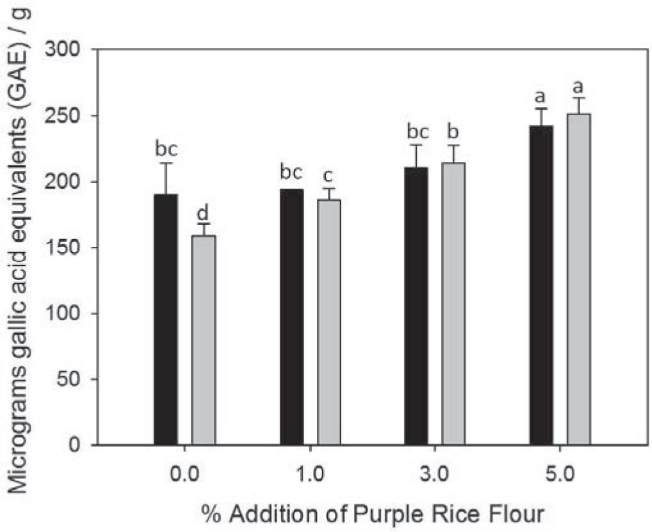

Figure 4 Total phenolic contents of yogurts made with addition of purple rice flour (PRF, ■), and combination of purple rice flour and corn starch (PRF+CS, ■).[a-d] Different letters were significantly different ($p < 0.05$).

5. Color

Color of yogurt samples reported in L*, a*, b* color space was shown in Table 2. Addition of PRF to yogurt changed the color of samples from off-white to purple due to phenolic compounds, mainly anthocyanin, containing in PRF. Decrease in L* (lightness) was observed when PRF was added, and no effect of CS on L* was detected. The a* increased with addition of PRF indicating that redness of yogurts was increased due to pigments in the PRF, while an increase in b* was observed when PRF was added.

Table 2 Color (L^* a^* b^*) of control yogurt (Control), yogurts made with addition of purple rice flour (PRF), yogurt made with 0.75% corn starch (CS), and yogurts made with addition of a combination of purple rice flour and 0.75% corn starch (PRF+CS).

Sample	L^*	a^*	b^*
Control	88.95 ± 0.17^{a}	-1.85 ± 0.05^{g}	11.35 ± 0.51^{a}
1% PRF	71.57 ± 0.75^{b}	4.42 ± 0.08^{e}	8.55 ± 0.16^{ed}
3% PRF	59.16 ± 0.69^{c}	6.57 ± 0.08^{b}	9.37 ± 0.09^{b}
5% PRF	50.97 ± 0.32^{d}	6.99 ± 0.08^{a}	8.19 ± 0.12^{d}
0.75% CS	88.42 ± 0.06^{a}	-1.63 ± 0.05^{f}	11.59 ± 0.17^{a}
1% PRF+0.75% CS	71.21 ± 0.34^{b}	4.58 ± 0.04^{d}	8.73 ± 0.06^{c}
3% PRF+0.75% CS	58.40 ± 0.16^{c}	6.37 ± 0.02^{c}	8.70 ± 0.02^{c}
5% PRF+0.75% CS	51.09 ± 0.50^{d}	7.04 ± 0.05^{a}	8.69 ± 0.08^{c}

[a-g]Different superscripts in the same column were significantly different ($p < 0.05$).

Conclusion and Discussion

This study demonstrated the possibility of using PRF as a promising stabilizer containing phenolic compounds. PRF could be added alone or in combination with conventional stabilizer to further enhance yogurt stability. Addition of PRF to yogurt premix samples significantly reduced fermentation time when incubated at 40°C. Improvement in physical properties was observed with reductions in spontaneous whey separation and syneresis and an increase in texture firmness when PRF was added. Addition of PRF in combination with CS reduced spontaneous whey separation and syneresis to a greater extent. An increase in firmness was probably due to depletion interaction between casein particles and polysaccharides, which enhanced formation of protein-protein bonds. Total phenolic contents of yogurts increased as the PRF was added. However, this findings require further study of consumer acceptability of PRF added yogurts.

Acknowledgements

The authors would like to thank Strategic Wisdom and Research Institute of Srinakharinwirot University for the funding of this project.

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