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Application of Functional Colorant Prepared from Black Rice Bran in Yogurt

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Abstract

Food color becomes a significant part of food products; not only for its appearance but also its attractive consumer acceptability. Black waxy rice is one of the most potential plant sources of dark purple color of anthocyanins. Therefore, the present study was conducted to prepare the functional food colorant from the black rice bran. The flavored yogurt was chosen in this study due to its low pH favors the bright color of anthocyanins. The colorant powder, obtained from this work, was dark purple in color and contained γ-oryzanol, total phenolic, and anthocyanins with the amount of 18.14 mg/g, 37.10 mg/g, and 9.48 mg/g, respectively. The colorant powder was indicated the successful application to yogurt. Yogurt added with colorant powder of 0.6% w/w presented a pleasant purplish-pink color (L = 68.41, C = 15.23). The chroma and lightness of yogurt were constant throughout 21 days under refrigeration storage (4°C).

Keywords: Black waxy rice bran; food colorant; yogurt; flavored yogurt

1. Introduction

Yogurt is a dairy product produced by bacterial fermentation of milk. Generally, the bacteria used to make yogurt are cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. In addition, *Lactobacillus acidophilus* and *bifidobacteria* are also applied to improve health benefits of yogurt as a probiotic source [1] and [2]. In recent years, the flavored yogurt has gained a lot of popularity. Basically, yogurt mixed with artificial flavors and colors, natural fruits with artificial flavors mixed, and natural fruit are commonly

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produced. The demand for natural food colorant is increasing because of awareness of positive health benefits of it. Therefore, the study on new natural sources of food colorants and their potential use as a functional ingredient is needed. Black waxy rice is one of the most potential plant sources of dark purple color of anthocyanin pigment. The bran layer of the rice kernel contains high level of bioactive compounds such as γ-oryzanol, anthocyanins, and phenolic compounds. A number of studies showed that these compounds can reduce low-density lipoprotein cholesterol (LDL), improve lipid profiles, have anti-inflammatory and anti-oxidative activities, may help to fight heart disease, and prevent diabetes [3]. Nevertheless, one limitation on application the anthocyanin pigment, as a food colorant in food products, is its poor stability [4]. The previous study by [5] optimized the natural food colorant processing using enzymatic extraction and maltodextrin to improve the stability of anthocyanin colorant extracted from black waxy rice. However, the study on the stability of this food colorant, when incorporated to food product, is considered necessary. Therefore, the present study was carried out to evaluate the potential use of colorant powder, prepared from black waxy rice bran, in yogurt.

2. Materials and Methods

2.1. Preparation of colorant powder from black waxy rice bran

Black waxy rice (*Oryza sativa* L.) paddy was purchased from local rice-milling factory in Mahasarakham province, Thailand. The rice samples were de-hulled and then polished (8% degree of milling) to obtain rice bran fraction. The black rice bran was extracted using enzymatic extraction, the method reported by [5] and [6]. The pH of the extracted solution was adjusted to 2.5 with 0.1 N HCl and filtered before the extract was mixed with maltodextrin (2g/100 ml) and then freeze dried (Heto PowerDry PL3000). The colorant powder of black rice bran *(CBRB)* was obtained and used for phytochemical analyses, color measurements, and yogurt preparation.

2.2. Color measurement

Color attributes of the samples was measured by Minolta Chroma Meter CR-300 (Konica Minolta, Japan). The color was expressed in L*, C*, h. The L* represents lightness (L* = 0 yields black and L* = 100 indicates diffuse white). The Chroma (C*) represents color intensity which is the distance of a color from the origin (a* = b* = 0) in a* and b* plane. Hue angle (h) expresses in degrees ranged from 0º to 360º, where 0º (red) locates on the +a* axis, then rotating anticlockwise to 90º (yellow) for the +b* axis, 180º (green) for -a* and 270º (blue) for -b*.

2.3. Determination the content of anthocyanins, total phenolic compounds, and γ-oryzanol

The black rice bran and the extracted powder were analyzed for anthocyanin content using the pH differential method [7]. Absorbance was measured at 520 nm with a UV-Vis. spectrophotometer (Shimadzu, Japan). The total phenolic compound content was determined using the Folin-Ciocalteu method [10]. The results of the total phenolic compounds were expressed as mg gallic acid equivalents (mg GAE) per gram of rice bran and freeze-dried powder.

The determination of γ-oryzanol was performed according to the method reported by [8] and [9] with some modifications. A reversed phase high performance liquid chromatography (RP-HPLC) was applied using HPLC system (model L-6200A) consisted UV–VIS photodiode array detector (Shimadzu, Japan). Separations were conducted with a Phenomenex C_{18}-reversed phase column (4.6×250 mm and 4 μm) (Phenomenex, USA).

Chromatography was performed at 45°C, injection volume of 20 µl and run time was 25 min. Gradient elution was performed with a mobile phase of methanol: butanol: water (92: 4: 4 (v/v) with flow rate of 1.0 ml/min, 12 min. The ratio of methanol: butanol: water was gradually changed to 92: 5: 3 (v/v) with the flow rate of 1.5 ml/min, 13 min. The γ-oryzanol was detected at 325 nm.

2.4. Yogurt preparation

Flavored yogurt was prepared by adding three different levels (0.2, 0.4 and 0.6% by weight) of the colorant powder to raw milk. Then the raw milk was pasteurized at 63ºC for 30 minutes and cooled down to 45ºC before a 0.02% (w/w) of the commercial probiotic dairy starter (ABT-5, Chr. Hansen's, Denmark) was inoculated. The yogurt was incubated at 40-42ºC until the pH of 4.5-4.6 was reached. The yogurt samples were stored at 4ºC for 21 days. For each sample group, three cups of yogurt were sampled randomly every 3 days until 21 days and used for color, pH, and total acidity determinations [11].

2.5. Statistical analysis

The mean values, standard deviations, and analyses of variance (ANOVA) were calculated using SPSS software (version 11). Means comparisons were performed using Scheffe's test. Significance of difference was defined at p <0.05.

3. Results and Discussion

3.1. Phytochemical contents and color of black rice bran and colorant powder (CBRB)

The contents of anthocyanins, γ-oryzanol, and total phenolic compounds in CBRB were 9.48, 18.14, and 37.10 mg/g, respectively (Table 1). It was about threefold increase in anthocyanin and γ-oryzanol content and tenfold increase in total phenolic compounds compared with those of untreated black rice bran. The anthocyanin content in black rice bran was close to that reported by [12], whereas the content of γ-oryzanol, and total phenolic compounds were closed to those studied by [13]. The CBRB obtained was dark purple in color, which had a darker shade compared with that of the bran (Table 1). This might be because of the higher level of anthocyanins in the powder.

Table1 Major phytochemical content and color of black waxy rice bran and colorant powder

Sample	Chemical compositions (mg/g)			Color		
	Anthocyanins	γ-oryzanol	Total phenolics	L*	C*	h
Black rice bran	3.31±0.04	7.00±0.04	3.86±0.02	38.95±1.17	13.80±0.12	11.37±0.35
Colorant powder	9.48±0.19	18.14±0.15	37.10±0.40	39.85±0.72	17.66±0.81	12.60±0.95

3.2. Total acidity (TA) and pH values of yogurt flavored by CBRB

The results revealed that the level of CBRB added to yogurt did not significantly affect the pH and TA of freshly prepared yogurt; the pH and TA values were 4.60 and 0.47%, respectively. However, after storage for 21 days, the pH and TA of all samples were gradually changed significantly to 4.37 for pH and 0.85% for TA as indicated in Fig 1 (0.6% CBRB) due to the lactic acid produced by the yogurt bacteria. These results were similar to that reported by [14].

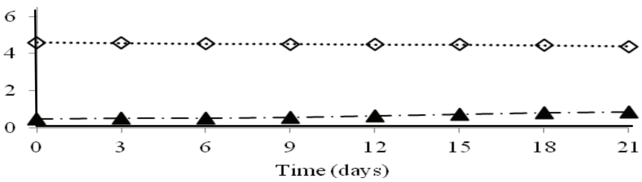

Fig. 1 Changes in pH values and total acidity of yogurt during storage: (···◇···) pH values and (·—▲—·) TA (%)

3.3. Color stability

The levels of CBRB added significantly affected the lightness (L*), chroma (C*), and hue angle (h) of yogurt (p<0.05). The yogurt was purplish pink in color depending on the levels of CBRB added. The CBRB adding of 0.6% showed the lowest lightness (68.42) and hue angle whilst its chroma (15.59) was strongest. After storage for 21 days, the color profiles of yogurt remained unchanged as indicated in table 2.

Table 2. Changes in lightness, chroma, and hue angle of yogurt during storage

Storage (days)	Levels of color powder addition (% w/w)			
	Lightness (L*)			
	0 (ns)	0.2 (ns)	0.4 (ns)	0.6
0	94.33±0.27^{d}	80.20±0.26^{c}	73.53±0.21^{b}	68.42±0.27$^{a, A}$
3	94.35±0.18^{d}	80.23±0.25^{c}	73.72±0.23^{b}	68.43±0.19$^{a, A}$
6	94.52±0.12^{d}	80.20±0.26^{c}	73.76±0.44^{b}	68.43±0.11$^{a, A}$
9	94.52±0.17^{d}	80.20±0.13^{c}	73.75±0.18^{b}	68.45±0.11$^{a, A}$
12	94.55±0.36^{d}	80.23±0.35^{c}	73.73±0.20^{b}	68.47±0.14$^{a, A}$
15	94.54±0.64^{d}	80.22±0.31^{c}	73.68±0.18^{b}	68.91±0.12$^{a, AB}$
18	94.56±0.13^{d}	80.25±0.21^{c}	73.75±0.12^{b}	69.39±0.28$^{a, B}$
21	94.55±0.41^{d}	80.26±0.09^{c}	73.80±0.05^{b}	69.45±0.37$^{a, B}$
	Chroma (C*)			
0	9.51±0.12^{a}	9.67±0.06^{a}	12.95±0.02$^{b, A}$	15.23±0.15$^{c, A}$
3	9.59±0.03^{a}	9.81±0.04^{b}	12.92±0.04$^{c, A}$	15.28±0.05$^{d, AB}$
6	9.48±0.04^{a}	9.83±0.13^{b}	12.96±0.03$^{c, A}$	15.55±0.06$^{d, AB}$
9	9.54±0.05^{a}	9.64±0.29^{a}	12.97±0.05$^{b, A}$	15.22±0.10$^{c, A}$
12	9.44±0.04^{a}	9.93±0.10^{b}	12.99±0.04$^{c, A}$	15.59±0.01$^{d, B}$
15	9.41±0.10^{a}	9.82±0.10^{b}	13.16±0.09$^{c, AB}$	15.41±0.08$^{d, AB}$
18	9.58±0.06^{a}	9.97±0.07^{b}	13.23±0.04$^{c, B}$	15.57±0.02$^{d, B}$
21	9.52±0.11^{a}	9.78±0.04^{a}	13.03±0.03$^{b, AB}$	15.51±0.16$^{c, AB}$
	Hue angle (h)			
0	108.83±0.77	18.34±0.52^{A}	12.65±0.20^{A}	8.73±0.31^{A}
3	108.40±0.70	19.58±0.16^{AB}	13.14±0.22^{ABC}	9.28±0.38^{A}
6	108.22±0.49	19.99±0.56^{BC}	13.08±0.41^{ABC}	9.67±0.13^{ABC}
9	108.08±0.95	20.88±0.66^{BCD}	13.92±0.10^{CDE}	10.51±0.11^{BC}
12	107.66±0.52	19.74±0.39^{AB}	12.92±0.20^{AB}	9.14±0.20^{A}
15	107.83±0.41	20.35±0.33^{BCD}	13.61±0.07^{BCD}	9.49±0.52^{AB}
18	106.97±0.38	21.25±0.17^{CD}	14.62±0.46^{E}	10.53±0.20^{C}
21	107.75±0.22	21.80±0.30^{D}	14.37±0.18^{DE}	10.61±0.24^{C}

^{a,b,c} Means within rows followed by the same letter are not significant different at $p < 0.05$; ^{A,B,} Means within columns followed by the same letter are not significant different at $p < 0.05$; ^{ns} Means within the same column are not significant different ($p \geq 0.05$)

When the lightness values were plotted against chroma values (Fig. 2 A) and the hue angle values were plotted against chroma values (Fig. 2 B). It was clearly observed that the lightness and chroma were not significantly changed with the increasing of the storage times. This might be due to the anthocyanins are stable in low pH and low temperature. Moreover, the shelf life of yogurt is short; this makes CBRB suitable for applying to flavored yogurt. The figures also show that the yogurt with different colorant concentrations had different shades of purplish pink color, which the yogurt added with 0.6% CBRB indicated the strongest color.

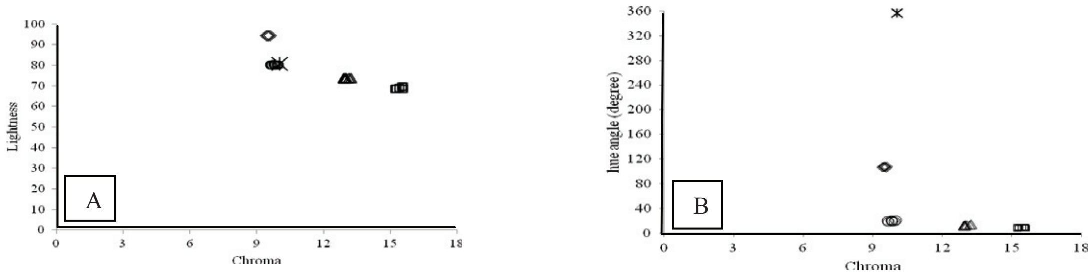

Fig. 2 Changes in hue angle and chroma (A) and lightness and chroma (B) of yogurt containing different levels of colorant powder during 21 days storage: (◊) control, (O) yogurt adding with 0.20% of freeze-dried powder, (Δ) yogurt adding with 0.40% of freeze-dried powder and (□) yogurt adding with 0.60% of freeze dried powder and commercial flavored yogurt (ж).

4. Conclusions

The colorant powder contained higher concentration of phytochemicals than untreated black rice bran. The successful application of colorant powder was achieved in terms of providing a purplish pink color to yogurt, having a good stability of color, and increasing phytochemicals contributed by black waxy rice bran. This makes the colorant powder possible to apply in food products as a functional food colorant. However, for further applications of this functional food colorant to other food products, more quality evaluations are required such as sensory and safety evaluation.

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