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Formulation Optimization of Purple Rice - Coconut Milk Drink Based on Physical and Chemical Properties using Mixture Design

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Abstract - This study aimed to determine the optimum formulation of purple rice - coconut milk drink. Extreme vertices design of mixture experiments for the three components was performed. The proportions of the three components were 20.00 - 28.78% boiled purple rice (BPR), 10.00 - 18.78% grated coconut meat (GCM) and 55.00 - 63.78% warm water at 80°C (WW) equivalent to a total of 93.78%. Four formulations were generated. The experimental data were analyzed using analysis of variance (ANOVA) and were fitted to a linear model equation. The results revealed that the increase in BPR produced a significant increase in antioxidant capacity, total phenolic content (TPC), total anthocyanin content (TAC), and the redness of the coconut milk drink. Besides, an increase of GCM caused greater values for TPC and lower value for a* value. The physicochemical properties of the drink were least affected by WW. The statistically significant predictive models with satisfactory coefficients of determination (R^2> 0.75) were used to plot the contour plot and determine the optimum values of responses. It was found that a formulation containing 28.78% BPR, 10.00% GCM, and 55.00% WW led to the desirable physical and chemical properties with combined desirability equals to 0.618. Confirmatory trial results achieved reasonably close between the observed and predicted values. The data presented could be useful in developing a plant - based functional drink for health - conscious consumers.

Keywords: coconut milk, black glutinous rice, mixture design, gum arabic

1. Introduction

Coconut milk is a milky white oil - in - water emulsion extracted from the grated coconut meat with or without added water and expelled most filterable fibers and residues (Chiewchan *et al.*, 2006). The main components of coconut milk are water and fat. However, the fats present are mostly in the form of medium - chain saturated fatty acids, predominantly lauric acid (Marina *et al.*, 2009). This fatty acid is less likely to clog arteries and has antiviral and antibacterial properties. Several Asian traditional foods, including Thai cuisines, use coconut milk as the main ingredient in home cooking (Simuang *et al.*, 2004). The food processing industries have also been prompted to develop new products from coconut milk. Several commercial products are made from coconut milk, such as spread, coconut syrup, coconut cheese, bakery products, and beverages. Coconut milk drinks prepared from coconut milk with or without coconut water, are thermally processed and sealed in a container to prevent spoilage.

The pigmented rice classified into black, red, and dark purple rice is considered an enriched source of nutrients and phytochemicals with high antioxidant capacity (Thitipramote *et al.*, 2016). Luem - Pua (*Oryza sativa* L.) is one of the indigenous pigmented Thai glutinous rice, which is consumed widely due to a pleasingly fragrant aroma and delicious chewy texture. The grain of this rice variety has a hard surface with a white inner area and has black pericarp primarily due to high amounts of anthocyanins, a group of reddish to purple water - soluble flavonoid. Recent studies reported that Luem - Pua rice contains a high content of phenolics, flavonoid, α - tocopherol, and resistant starch (Praman *et al.*, 2018). These phytochemicals are believed to exhibit the important roles for protection against oxidative damages implicated in a range of diseases, including cancer and cardiovascular diseases (Ciulu *et al.*, 2018).

The mixture design is a class of the response surface experiments in which the independent variables are the proportions of ingredients or components under investigation and usually sum to 100%. The dependent variable (measured response) is assumed to depend only on the proportions of the mixture components (Goupy and Creighton, 2006). The mixture design studies aim not only to develop better or innovative formulations of the product, but also to create general conceptions about responses and interactions between independent factors (Maia *et al.*, 2011).

The market for functional beverages with a high content of bioactive compounds has sharply grown due to the current trend of consumers seeking ready - to - consume, healthy, and nutritionally balanced diets. Coconut milk is the milk alternative with a texture closest to that of whole milk and its benefits such as free - from cholesterol and high amounts of lauric acid (Market Research Future, 2020). Rice milk is also dairy alternatives with no cholesterol and lactose. This type of product is high in carbohydrates and very low in protein when compared with dairy milk (Yigita, 2019). Both types of milk are primarily produced with added fortified elements e.g. calcium to overcome the lack of nutrients in it. In Thailand, coconuts and pigmented rice are available in abundance. A lot of commercial products from coconut milk or rice milk, but pigmented rice - coconut milk drink is still rare in the market. This study is interested in the production of ready - to - drink coconut milk with the addition of Luem - Pua rice to increase the number of bioactive compounds. The target market for this product is the vegan and lactose intolerant population. To produce a stable product, gum arabic is added to the extract obtained from coconuts and rice. Gum arabic is a complex mixture of polysaccharides and glycoprotein, mainly consisting of arabinose and galactose. These complex carbohydrates can adsorb at the oil in water interface, reduce the surface tension, interact with the starch and protein, and modify the crystallization of fat and oil (Smith, 1991). By FDA standards, it is generally regarded as safe (GRAS) with no upper limit as a food additive. In a previous research by Onuegbu *et al.* (2015), the stability of the coconut - carrot drink was well improved by 4% gum arabic addition. The objectives of this study, therefore, were to obtain the optimum ratios of boiled purple rice (BPR): grated coconut meat (GCM): warm water (WW) for the production coconut milk drinks with high antioxidant activity and to investigate its effects on the physical and chemical properties of the drinks

2. Materials and Methods

2.1 Plant materials and chemicals

Freshly GCM, sugar, and Luem - Pua glutinous rice were purchased from the local market in Mahasarakham province, Thailand. Food grade gum arabic was obtained from Chemipan Ltd., Thailand. Folin - Ciocalteau reagent, gallic acid, 2, 2′ - azinobis (3 - ethylbenzothiazoline - 6 - sulfonic acid) diammonium (ABTS) and 2,2 - diphenyl - 1 - picrylhydrazyl (DPPH) were bought from Sigma Chemical Co. (St. Louis, MO). All other reagents and solvents used were of analytical grade.

2.2 Production of purple rice - coconut milk drink

Purple rice - coconut milk drink was prepared using the method described by Onuegbu *et al.* (2015) with some modification. Briefly, 50 g Luem - Pua rice was boiled in 500 mL drinking water (ratio 1:10 w/v) with intermittent stirring for 20 min and left until cooled. BPR, GCM, and WW at 80°C were weighed out and were mixed at a varying ratio. After 3 min of steeping, the mixture was crushed in an electric blender at maximum speed for 2 min. The obtained suspension was sieved through a strainer and filtered with a muslin cloth (pore size 2 mm) twice. Subsequently, the filtrate was heated to 70°C and added with 3.39% of sugar and 2.83% of gum arabic. The resultant mixture was homogenized using a Nissei AM - 8 homogenizer (Nihonseiki Kaisha Ltd, Tokyo, Japan) at a speed of 50,000 rpm for 10 min. The product was hot - filled into 180 mL sterile glass bottles. In - container pasteurization of rice - coconut milk was done in a thermostatic water bath (model UM - SW30L, UMAC, Thailand) at 70°C for 15 min. The temperature of water bath was carefully maintained above 72 ± 1°C. The pasteurized drink was cooled under ambient temperature and immediately down to 4°C. The product was kept at 4°C for 7 days before analyses. The viable plate count of all the samples was within the acceptable standard (shall not over 10^5 cfu/mL) of Asian and Pacific Coconut Community (Tarek *et al.*, 2020).

2.3 Experimental design

There were five components: BPR, GCM, WW, white sugar, and gum arabic used for the production of coconut milk drink. The present study employed a mixture experiment with three components. The proportions of BPR (A), GCM (B), and WW (C) were restricted to be 20.00 - 28.78%, 10.00 - 18.78%, and 55.00 - 63.78%, correspondingly and were summed to 93.78%. In contrast, the contents of sugar and gum arabic were fixed at the rate of 3.39% and 2.83%, respectively. The constraints (minimal/maximal) for each component were established from the coconut - carrot drink formulation of Onuegbu *et al.* (2015), consisting of 250 g coconut, 250 g carrot, and 500 - 700 mL water at 50°C. A mixture design was carried out using Design Expert (v. 7.0, Stat - Ease Inc., Minneapolis, MN, USA). Three vertices of the triangle points and one centroid (inside the triangle) ascribed to the ternary combinations are obtained. The complete experiment design was shown in (Table 1). Each experimental formulation was performed two times to design a robust model.

Table 1. Formulation of purple rice - coconut milk drinks used in the study

Runs	Proportions of components (%)				
	A: BPR	B: GCM	C: WW	Sugar	Gum arabic
1	20.00	10.00	63.78	3.39	2.83
2	28.78	10.00	55.00	3.39	2.83
3	20.00	18.78	55.00	3.39	2.83
4	22.93	12.93	57.92	3.39	2.83

Where: (low) 20.00 < A < 28.78 (high); (low) 10.00 < B < 18.78 (high); (low) 55.00 < C < 63.78 (high): and A+B+C = 93.78% of total weight.

2.4 Determination of total solids, pH viscosity and color

The total solid was determined by drying the samples at 105 ± 2°C (AOAC, 2000). The pH was measured using a pH meter (Five easy F20; Mettler Toledo). The viscosity was determined at 25°C using Brookfield DV - II+ viscometer with spindle no.1 at 100 rpm. The color was measured using a Minolta Color Meter (Chroma Meter CR - 400, Minolta, Japan). The average color values of L* (lightness - darkness), a* (red - green) and b* (yellow - blue) were recorded. Hue angle and chroma were calculated from a* and b* using the following formulas:

$$\text{Hue angle } (h_{ab}) = \tan^{-1} (b^*/a^*)$$

$$\text{Chroma } (C^*) = (a^{*2} + b^{*2})^{0.5}$$

2.5 Determination of drink stability

The drink stability was measured by the method previously described by Onuegbu *et al.* (2015) with slight modification. Briefly, the drink samples (60 mL) were poured into 100 mL graduated cylinders and kept undisturbed in the refrigerator at 4°C. The height of the drink was initially recorded. Changes in drink stability were indicated by separation into two layers. The height of the upper layer was subsequently recorded after 72 h of storage. The stability (%) was calculated as follows: (height of upper layer /height of the drink) × 100.

2.6 Determination of total phenolic and total anthocyanin contents

The total phenolic content (TPC) was spectrophotometrically determined using the Folin - Ciocalteu method (Singleton *et al.*, 1999) and was expressed as mg gallic acid equivalents per 100 mL (mg GAE/100 mL). Total anthocyanin content (TAC) was determined using a pH - differential spectrophotometry method (AOAC, 2000), and the result was expressed as mg cyanidin - 3 - glucoside/100 mL.

2.7 Determination of antioxidant activity

The total antioxidant capacity was measured using ferric reducing power assay (FRAP) and free radical scavenging activity was evaluated using ABTS and DPPH radicals.

The ABTS assay was followed the method of Re *et al.* (1999) with a slight modification. The $ABTS^+$ solution was prepared by mixing a 7.0 mM ABTS solution with 2.45 mM potassium persulfate solution (2:1 v/v). The mixture was incubated in the dark for 12 h and then diluted in methanol until the absorbance value of 0.700 ± 0.02 at 734 nm was reached. A diluted test sample (0.5 mL) was mixed with 4.5 mL of prepared $ABTS^+$ solution. The absorbance of the mixture was measured at 734 nm using a spectrophotometer after 6 min. The control was conducted in the same manner, but methanol was used in place of the sample. The DPPH assay, the procedure described by Alyaqoubi *et al.* (2015) was followed with a slight modification. In brief, a diluted sample (1 mL) was mixed with 6 mL of 0.06 mM DPPH in absolute ethanol, and allowed to stand at room temperature in the dark for 30 min. The absorbance of the mixture was measured at 517 nm using a spectrophotometer. The control was conducted in the same manner but absolute ethanol was used instead of the sample. ABTS and DPPH radical scavenging activities were calculated as follows: Scavenging (%) = $[1 - (A_{sample} - A_{control})] \times 100$, where A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the control.

The FRAP was performed according to the method described by Benzie and Strain (1992). The fresh working FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6) with 10 mmol/L TPTZ (2, 4, 6 - tripyridyl - s - triazine) in 40 mmol/L HCl and 20 mmol/L $FeCl_3$ in a ratio of 10:1:1 (v/v/v). A diluted sample (120 µL) was mixed with 3.6 mL of FRAP reagent and 360 µL of distilled water. After 4 min of incubation at 37°C, the resulting intense blue coloration was measured at 593 nm. Aqueous solutions of known $FeSO_4.7H_2O$ concentration were used as standards. The data is shown as FRAP values (mmole/100 mL Fe (II).

2.8 Statistical analysis

Data were statistically analyzed using SPSS 14.0 software (SPSS Inc., Chicago, IL, USA) and were expressed as means ± standard deviation (SD) of duplicate experiments with triplicate analysis. Duncan's Multiple Range Test (DMRT) was performed for mean comparison when ANOVA showed significant differences at the 95% confidence level.

The effect of components on the physical and chemical properties of purple rice - coconut milk drink was analyzed. Data were fitted to a linear model applying the least squares regression method to estimate the coefficients in Eq. 1:

$$Y_i = b_1A + b_2B + b_3C \quad \text{(Eq. 1)}$$

where Y_i is the predicted response, b_1 b_2 and b_3 denoted the magnitude of the effect of each component (regression coefficients) and A, B and C represent the proportions of the component (independent variable) (Yolmeh *et al.*, 2017). The models were subject to ANOVA to determine the significance ($P < 0.05$) and coefficient of determination (R^2). When selecting the appropriate statistical model, the following parameters: standard deviation, p - values, and R - square adjusted (R^2 adj) were considered. The goodness of the model was also checked using a lack of fit test.

After model fitting was performed, significant dependent variables were used to predict optimal purple rice - coconut milk drink formulation with the goal to maximizing ABTS radical scavenging activity, TPC, TAC, a* and C* values of the drink. The selection of appropriate component levels was considered from the highest value of desirability (0.0 for undesirable to 1.0 for very desirable). The percentage error measured between predicted and experimental values were calculated to validate the model's response output. All statistical calculations, mixture design, generation of response surfaces, optimization, contour plots were carried out using Design Expert (v. 7.0, Stat - Ease Inc., Minneapolis, MN, USA).

3. Results and Discussion

3.1 Fitting for the best model

(Table 2) showed the results of mixture design studies. After fitting the independent and dependent variables to models, the best model was selected using the following guides: low standard deviation, high predicted R^2 (Cornell, 2002). According to (Table 3), the regression models of ABTS radical scavenging activity, TPC, TAC, C* and a* were adequately predicted experimental results with R^2 of more than 0.75. Significant probability values ($P < 0.01$) and non - significant lack of fit values ($P > 0.05$) of these responses were also observed, indicating that most variations could be well explained by the proposed model (Myers and Montgomery, 2002). In cases of FRAP value, b*, H_{ab}, total solids, pH, viscosity and stability, responses were inadequately fitted to the linear models because of R^2 smaller than 0.75. DPPH and L* models fitted inadequately, showing significant lack of fit ($P < 0.05$).

Table 2. Physical and chemical properties of purple rice - coconut milk drinks

Properties	Runs			
	1	2	3	4
TAC (mg cyanidin - 3 - glucoside/100 mL)	0.42±0.06^{c}	1.13±0.06^{a}	0.71±0.07^{b}	0.80±0.07^{b}
TPC (mg GAE/ 100 mL)	341.1±13.1^{c}	381.4±18.5^{b}	412.8±12.4^{a}	380.6±19.0^{b}
ABTS (%)	72.03±2.39^{b}	82.30±3.45^{a}	72.22±2.60^{b}	75.78±0.56^{b}
DPPH (%)	52.07±0.54^{c}	60.11±0.87^{a}	53.50±0.46^{c}	57.68±2.03^{b}
FRAP (mmole Fe (II)/100 mL)	2.31±0.31^{a}	2.66±0.30^{a}	2.80±0.19^{a}	2.64±0.35^{a}
L*	56.03±0.61^{b}	54.10±0.69^{c}	58.41±0.41^{a}	55.57±0.32^{b}
a*	1.41±0.27^{c}	2.64±0.38^{a}	1.16±0.38^{c}	1.88±0.28^{b}
b*	6.04±0.04^{a}	6.25±0.20^{a}	6.30±0.04^{a}	6.22±0.27^{a}
H_{ab} (°)	76.85±2.52^{ab}	67.13±3.63^{c}	79.57±3.41^{a}	73.11±3.02^{b}
C*	6.21±0.05^{c}	6.80±0.05^{a}	6.42±0.05^{b}	6.50±0.18^{b}
Total solids (%)	17.37±0.34^{b}	17.52±0.36^{b}	20.95±1.45^{a}	17.98±1.58^{b}
pH	5.43±0.01^{b,c}	5.38±0.05^{c}	5.51±0.05^{a}	5.46±0.01^{ab}
Viscosity (cP)	45.0±17.6^{a}	67.7±21.4^{a}	48.7±18.4^{a}	46.6±18.1^{a}
Stability (%)	0.92±0.01^{b}	1.39±0.55^{ab}	1.77±0.51^{a}	1.01±0.03^{b}

1. Results shown are mean ± SD of two experiments each with triplicate analyses (n=6).
2. Different letters in the same row differ significantly at P < 0.05 by DMRT.

3.2 Total anthocyanin content

The highest BPR proportion and lowest WW proportion (formulation 2) resulted in the highest TAC (1.13 mg cyanidin - 3 - glucoside/100 mL). The results were in line with the redness (a* and H_{ab}) of the product (Table 2). The lowest TAC (0.42 mg cyanidin - 3 - glucoside/100 mL) occurred in formulation 1 with the lowest BPR proportion and the highest WW proportion. The linear regression model was highly significant (P < 0.01) with high determination coefficients (0.8639). The BPR (A) had a notable influence on anthocyanin (P < 0.001) as shown by the higher coefficient value (Table 3). This was due to high amounts of anthocyanins accumulated in the pericarp layer of Leum Pua grains. The higher the amount used, the higher the TAC. In contrast, a large amount of added WW (C) decreased the TAC and the redness of the drink. The extraction rate of anthocyanins depends on the pH - value, liquid - solid ratio, extraction time and temperature, and other factors (Li *et al.*, 2019). When the amount of extraction solvent is increased within a certain range, more bioactive compounds can come into contact with the solvent leading to higher leaching rates (Mohamad *et al.,* 2013). But anthocyanins are heat sensitive, and they start to degrade rapidly above 60°C (Pragalyaashree *et al.*, 2018). Increasing proportion of WW at 80°C may probably cause degradation of anthocyanin due to too high a temperature accumulation. The results were similar to those of Muangrat and Saengcharoenrat (2018). From the contour plot (Figure 1A), the nearer points to the BPR (A) vertex represented the higher values of anthocyanin

3.3 Total phenolic content

The highest TPC (412.80 mg GAE/100 mL) was obtained for formulation 3 with the highest amount of GCM (Table 2). The lowest TPC (341.1 mg GAE/100 mL) was obtained for formulation 1 with the lowest amount of GCM and BPR. According to (Table 3), the phenolic response was fitted to the linear model with high determination coefficients of 0.7669. GCM had the most significant influence on TPC, followed by BPR. This was supported by the fact that the coconut milk contained a higher amount of phenolic compounds than Luem - Pua rice (575.15 mg GAE/100 g vs. 163 ± 3 mg GAE/100 g (Alyaqoubi *et al.*, 2015; Jansom *et al.*, 2017). In the contour plot (Figure 1B), the closer points to GCM (B) vertex represented the higher TPC.

In comparison to the study of Alyaqoubi *et al.* (2015), the purple rice - coconut milk drink possessed lower TPC than coconut milk, goat milk and cow milk (385.31 vs. 477.68 - 575.15 mg/ 100 mL). However, the coconut milk in the present study was relatively a richer source of TPC compared with those of fresh fruit juices, commercial 100% fruit juices and fruit drinks in the study of Wern *et al.* (2016) which were at the ranges of 13.38 - 80.40, 21.65 - 130.39 and 3.32 - 45.10 mg GAE/100 mL, respectively.

Table 3. ANOVA of the regression models and regression coefficients for significant physical and chemical parameters of purple rice - coconut milk drinks

Variables (Yi)	Independent variables			R^2	p (model)	p (lack of fit)
	A	B	C			
TAC	0.064***	0.016	- 0.016*	0.86	**	0.71^{ns}
TPC	6.598***	10.168***	1.649***	0.77	***	0.75^{ns}
ABTS	1.687***	0.539***	0.517***	0.79	***	0.86^{ns}
DPPH	1.264***	0.512***	0.349***	0.81	***	0.003
FRAP	0.050***	0.065***	0.011	0.30	*	0.73^{ns}
L*	0.409***	0.881***	0.609***	0.86	***	0.003
a*	0.128***	- 0.040*	- 0.011	0.76	***	0.37^{ns}
b*	0.080***	0.086 ***	0.056***	0.27	*	0.84^{ns}
H_{ab}	- 0.088	1.33***	1.02***	0.71	***	0.36^{ns}
C*	0.116***	0.073***	0.049***	0.84	***	0.57^{ns}
Total solids	0.154*	0.545***	0.136**	0.68	***	0.34^{ns}
pH	0.053***	0.067***	0.058***	0.64	**	0.36^{ns}
Viscosity	0.594	1.071*	0.524*	0.04	ns	0.96^{ns}
Stability	0.031	0.110***	- 0.013	0.74	**	0.06^{ns}

Where, A= boiled purple rice, B = grated coconut meat and C = warm water; ns = not significant. *, ** and *** are significance levels at $P < 0.05$; $P < 0.01$; and $P < 0.001$.

3.4 Antioxidant activity

Three assays were analyzed to measure the antioxidant activity, i.e. DPPH, FRAP, and ABTS. The FRAP values of all drinks were comparable (P > 0.05). By ABTS and DPPH assays, the highest inhibition percentages (82.30 ± 3.45% and 60.11 ± 0.87%) were given to the formulation 2 in which the BPR was present at the highest concentration and WW was present at the lowest concentration. Formulations 1, 3, and 4 exhibited a similar ability to scavenge ABTS and DPPH radicals, varying from 72.03% to 75.78% and 52.07 to 57.68%, respectively (Table 2).

From the results in (Table 3), we found no linear correlation between the FRAP values and predicted variables in the response with low R^2 of the fitted model (0.2988). BPR, GCM and WW showed a significant positive effect on ABTS and DPPH scavenging activities of the drinks. BRP displayed a greater effect than other components. The contour plot confirmed that antioxidant activity increased toward the BPR (A) vertex (Fig. 1C). The contribution of antioxidant activity in BRP could be due to anthocyanin. It is reported that ethanol extract of Leum Pua presents anthocyanins in concentration of 17.784 mg/mL extract (Thitipramote *et al.*, 2016). The phenolic compounds could be associated with the antioxidant capacity of GCM. Ngampeerapong and Chavasit (2019) reported the TPC of coconut meat from different sources i.e. Thailand, Indonesia and Vietnam in the range of mg 6,391 - 10,342 mg GAE/100 g. These values were 39 - 63 times more than in Luem - Pua rice reported by Jansom *et al.* (2017). Since most phenolic compounds are naturally hydrophilic, the higher amount of WW would positively influence on antioxidant power of the coconut milk drink.

Pearson's correlation analysis was performed to evaluate the relationship between the antioxidant assays and bioactive compound contents (data not shown). ABTS and DPPH radical scavenging activities showed a significant positive correlation (r = 0.811; P = 0.000). This is correspond with the research findings of Alyaqoubi *et al.* (2015) that shown a highly positive correlation between FRAP, TPC, and DPPH assays of the tested milks. TAC *was* significantly correlated (P = 0.004) to *DPPH* (r = 0.876), and weakly correlated (P = 0.059) to ABTS (r = 0.688). TPC was positively and strongly correlated with FRAP (r = 0.621, P = 0.010), but no relationship was found between TPC versus DPPH (r = 0.196) and ABTS (r = 0.172) assays. This indicated that anthocyanin and phenolic compounds were the main contributor to antioxidant activity in terms of radical scavenging and iron ion reducing ability, respectively. However, there was insignificant correlation between TAC and TPC (r = 0.513, P = 0.194). The antioxidant activities of coconut milk drink could not be entirely predicted on the basis of its TPC and TAC. Phytic acid, γ - oryzanol, tocopherols, and tocotrienols in rice grains (Goufo and Trindade, 2014) also partially contribute to antioxidant functions which were not quantified in the present study.

3.5 Color values

Formulation 2 had the highest a* and C* values, but the lowest L* and H_{ab} values as compared to other formulations (P < 0.05). Meanwhile, no significant differences in b* values between the coconut milk drinks were observed, ranging between 6.04 and 6.30 (Table 2). H_{ab} values near 90° relate to yellow color, while values closer to 0° relate to very red samples. Chroma is a measure of the color saturation (Slesinski, *et al.*, 2000). In our study, the small variation of chroma values occurred, although significant difference was found. Therefore, H_{ab} could be a good measure of the red color. From the regression coefficients of equations shown in (Table 3), BPR was the term that most affect the a* and C* values. Formulation 2 containing a high BPR level, therefore, appeared a redder color than the others. Conversely, for the sample containing either high GCM or high warm water levels, the L* and H_{ab} values were high, and the a* value was low (negative regression coefficient). The color of formulations 1, 2 and 4 seemed more yellowish - white than formulation 2. The R^2 values of the predicted models for a* and C* were higher than 0.75, demonstrating relative adequate for the prediction purpose. Contour plots in (Fig. 1D and 1E) illustrated the effects of three components on the color parameters of coconut milk drinks. It was indicated that the a* and H_{ab} values increased toward the BPR (A) vertex.

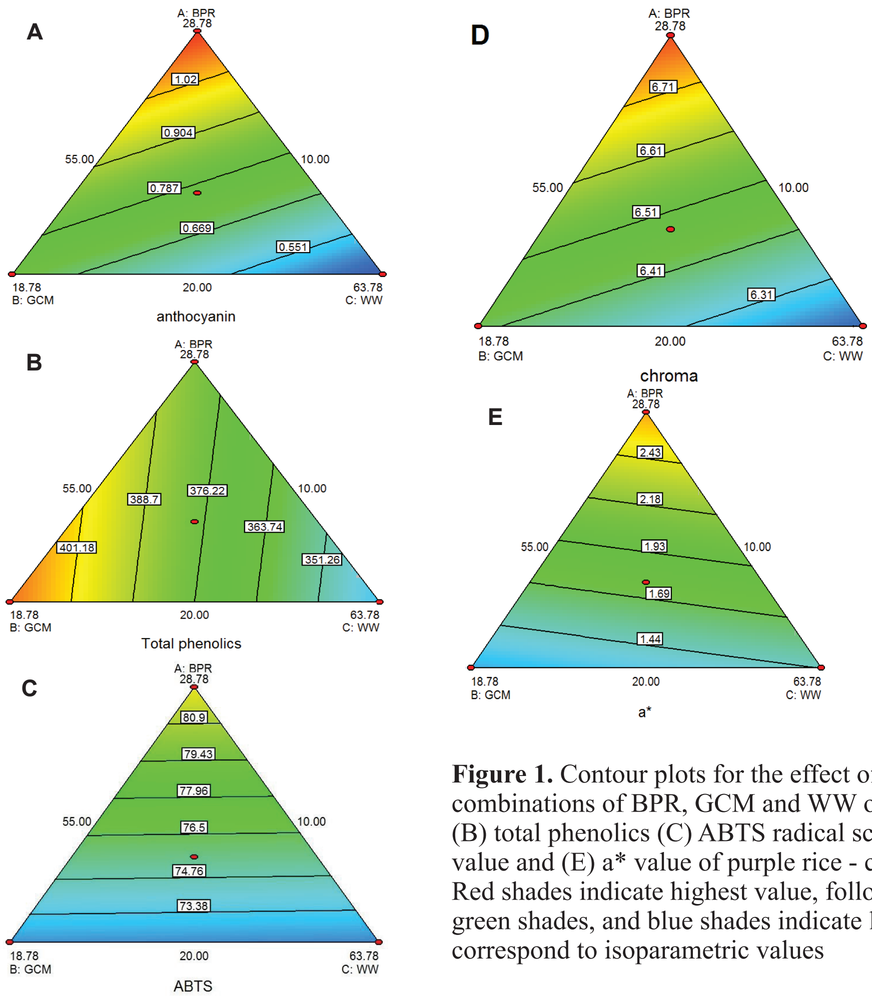

Figure 1. Contour plots for the effect of different combinations of BPR, GCM and WW on (A) anthocyanin (B) total phenolics (C) ABTS radical scavenging (D) C* value and (E) a* value of purple rice - coconut milk drinks. Red shades indicate highest value, followed by yellow and green shades, and blue shades indicate lowest value. Lines correspond to isoparametric values

3.6 Mixture optimization

The optimal formulation of purple rice - coconut milk drink was obtained by mathematical solving the regression equations (Table 3). With the goal of highest ABTS scavenging capacity, TPC, TAC, a* and C* values, a set of combinations of BPR, GCM and WW (A, B, and C) was given by the software. According to the fitting performed, the coordinates corresponding to the best desirability value (0.618) were 28.78%, 10.00%, and 55.00% for A, B, and C, respectively. The predicted values of ABTS scavenging capacity, TPC, TAC, a* and C* to this point were 82.37%, 382.23 mg GAE/100 mL, 1.14 mg cyanidin - 3 - glucoside/100 mL, 2.67 and 6.80, correspondingly. (Table 4) showed the performance of the model indices. The models of all responses showed a good fit with the small error (less than 10%).

Table 4. Optimized formulations and predicted responses based on constraints applied to significant variables compared to experimental data

Variables	Optimization criteria	Optimized formulation	Experimental formulation	
Independent				
A: boiled purple rice	Range: 20 - 28.78	28.78	28.28	
B: grated coconut meat	Range: 10 - 18.78	10.00	10.50	
C: warm water	Range: 55 - 63.78	55.00	55.00	
Total (A+B+C)	93.78	93.78	93.78	
Dependent			Predicted values	Experimental values
ABTS (%)	Maximized	82.37	81.80	81.80
TPC (mg GAE/ 100 mL)	Maximized	382.23	384.05	382.32
TAC (mg cyanidin - 3 - glucoside/100 mL)	Maximized	1.14	1.10	1.04
a*	Maximized	2.67	2.59	2.67
C*	Maximized	6.80	6.74	6.66

4. Conclusions

A mixture design was found to be an effective technique to optimize the inclusion of BRP, GCM, and WW in the purple rice - coconut milk drink. The results indicated that the use of BRP could significantly elevate antioxidant power, TPC, TAC and a* value of the drink, while an increase of GCM had led to greater values for TPC. The optimized formulation was as follows: 27.78% BRP, 10.00%, GCM and 55.00% WW. The experimental results of the optimized are consistent with the predicted values. Future studies will be conducted to examine the optimum level of other formulation ingredients e.g. sugar and stabilizer.

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6. References

Degradation kinetics of anthocyanin extracted from roselle calyces (Hibiscus sabdariffa) Degradation kinetics of anthocyanin extracted from roselle calyces (Hibiscus sabdariffa)

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