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RESPONSE SURFACE OPTIMIZATION OF ENZYMATIC HYDROLYSIS OF PURPLE RICE (CULTIVATED IN SOC TRANG, VIETNAM) USING AMYLASES AND FORMULATION OF HEALTHY RICE MILK

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Abstract
The purple rice (ST variety) which was cultivated in Soc Trang province, Vietnam was used for this research. The rice starch was hydrolysed by two-step enzymatic using α-amylase and gluco-amylase for making rice milk. The effects of enzyme concentration and time on hydrolysis efficiency (total soluble solid content - TSS) were investigated. The Central Composite Design and Response Surface Methodology were used for the experimental design and results analysis. To improve the quality of rice milk, the effect of five formulations mixture with various milks on sensory attributes and nutritional content were measured. The method of Principle Component Analysis and Check-all-that-apply analysis were applied. The results showed that the optimum enzyme concentration and time ofα-amylase (0.149% and 57 minutes) and gluco-amylase (0.143% and 40.94 minutes) hydrolysis were found as R^2 were 0.88 and 0.97, respectively. The maximum TSS production at optimum condition was 16%. For purple rice milk production, formula 4 (70% rice milk, 10% soybean milk, 12% water caltrop milk and 8% sesame milk) was presented better sensory quality than other formulations. The content of protein, carbohydrate, lipid and anthocyanin content of final product were 1.85%, 7.3%, 1.32% and 2.89 mg/L, respectively.
Keywords*:* healthy rice milk, enzyme hydrolysis, nutrient, formulation, sensory characteristics.

1. Introduction
Rice (*Oryza sativa*) is the largest staple foods for almost half of the population in the world (Folorunso, 2016), it is considered as a basic source of energy as human food. The majority of rice production and consumption are among Asian countries. More than 2 billion people obtain 60-70% of their daily calories intake from rice. From the nutritional standpoint, rice provides several components such as main of carbohydrates, source, protein, fats, fiber, minerals and also vitamins (Folorunso, 2016). It has high digestibility, biological value and protein capability amount owing to the presence of high concentration of lysine over than all the cereal. The varieties of rice that differ in grain color are more popular for the human consumption in these days due to their benefits for health. There are many special cultivars if rice that contains color pigments such black or purple rice, brown rice, red rice. The purple rice has characteristic as a deep purple grain which containing large amount of antioxidant properties (Settapramote *et al.*,2018). The kernel of this rice color is deep purple or black due to a pigment known as anthocyanin. The key portion in anthocyanin is cyadinin and peonidin which is found in a pigment substance on the surface layer of rice grain (Leardkamolkarn *et al*., 2011). Consider as the source of antioxidants that have the ability to inhibit the formation or reduce the concentrations of reactive cell damaging free radicals and prevent the heart disease. The health benefits of this type of rice have recently been reported by several investigators. A recent report indicated that anthocyanin supplementation in humans improves LDL and HDL level and able delay cancer development (Thomasset *et al*., 2009), improve lipid profiles and prevent diabetes. Moreover, the major nutrient component is starch comprised of amylose (13-30%) and amylopectin (with a dominant amount of 70-85%). While amylose is a linear chain made up of hundreds of glucose molecules that link by α 1,4-glycosidic linkages, amylopectin has a larger molecule consisting of α-glucan chains joined by frequent α 1,6 branch point. Enzymatic hydrolysis of rice starch into soluble sugar is a potential process leading to the processing efficiency of rice production as rice slurry.

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α-amylase (1,4- α-D-glucan glucohydrolase) to produce maltodextrins which then is saccarified by gluco-amylase (1,4- α-D-glucan glucohydrolase). It is well know that α-amylase is endo-amylases catalyzing the hydrolysis of internal α-1,4-glucosidic linkages in starch polysaccharides. While gluco-amylase is exo-amylase and responsible for hydrolyzing both α-(1-4) and α-(1-6) glycosidic linkages in liquefied starch during sacchrification gluco-amylase reduce glucose molecules in a stepwise manner from the non-reducing end of the substrate molecules (Novozymes, 2012).
The combination of α -amylase and gluco-amylase in hydrolysis has been reported in many authors. The optimization of some parameters and the knowledge of the interactions between these variables are important for the successful economical production of enzymes and determination of its industrial applicability. RSM is the statistical method has been used to optimize the effect of process parameters for enhanced production and yields of many target products (Claver *et al.*, 2010). Thuy *et al.* (2015) studied the hydrolysis rice starch by two-step enzymatic had significantly affect higher the maximum DE index 77.382% could be obtained at optimal conditions at temperature 60.39% in 210 min and gluco-amylase dose of 0.077%. Deswal *et al.* (2014) studied the optimization of enzymatic using RSM. The simultaneous effect of slurry concentration (22-35% w/w), enzyme concentrations (0.5-2.5% w/w) and liquefaction time (30-90 min) on the yield, total solids and rheological parameters of oat milk. The results showed that the optimum conditions for making oat milk were: 27.1% w/w slurry concentration, 2.1% w/w enzyme concentration and liquefaction time of 49 min.

However, in these days the rice production remains less profitable due to its unstable market price, less competitively quality and the lack of values-added processing. Therefore, product diversification is an important solution to improve the value of rice production. Rice milk is used to replace cow milk in the diet by increasing the amount of consumers for medical reasons such as lactose intolerance, cow's milk allergy. It is considered the best hypoallergenic form of milk(Gajdoš Kljusurić *et al*., 2015). Meanwhile, chemical compound of rice milk was made from rice has high carbohydrate and sugar but low in protein, fat and saturated fat (Perezgonzalez, 2008). To enhance the nutrient of rice milk and make the product completely of nutrient sources, it is able to mix with various ingredients such as soymilk, sesame milk and water caltrop slurry. In this study, the two important process parameters were investigated. Firstly, the part of hydrolysis starch by enzymes (α-amylase and gluco-amylase) the concentration of enzyme and time affecting the performance of the enzymes were subjected by the optimization using Response Surface Methodology (RSM) using the central composite design (CCD) model. Secondly, this work was studied the effect of mixing various levels soy, sesame or water caltrop milk with rice milk. The analysis of chemical composition and sensory evaluation of products were performed.

2. MATERIALS AND METHODS

2.1 Materials

Rice (ST Purple rice) purchased from SocTrang province (Vietnam), soybean (MTD 760 variety) supplied from department of genetic and plant breeding, College of Agriculture, Can ThoUniversity, sesame and water caltrop were bought in the local market of Can Tho city. Enzymes used in this study were obtained from Novozyme Biology Co.Ltd, (Denmark).

2.2 Method

Enzymatic hydrolysis of rice starch

Rice starch was blended into a puree. The ratio between rice and water is 1:7, and then gelatinized by boiling at temperature 85^{o}C for 30minutes; the puree was liquefied by α-amylase at 80^{o}C with α-amylase concentration between 0.05÷0.2%, with the hydrolysis time range of 35÷50 minutes. Then the puree was saccharified by gluco-amylase at 60^{o}C, with the dose of 0.05 ÷0.2% and saccharification time from 35 ÷50 mintues. After hydrolysis, the suspension was filtered through 2 layer fabric. The filtered hydrolyte solution was adjusted and mixture with other milk to enhance nutrient and sensory properties of the products.

Preparation of milk formulations

The preparation of soymilk, sesame milk and water caltrop milk: soy milk and sesame milk were prepared as describes by Fitrotin *et al*. (2015). Water caltrop milk was prepared by peeling and cleaning, and then blended with the electric blender. The sample hydrolyzed the starch by using two enzymes: α-amylase and gluco-amylase with the optimal condition that applied in hydrolysis rice starch. After obtained all of the milk was mixed into various formulations presented in Table 1. Rice milk determined the chemical properties and sensory evaluation to indicate the highest nutrient formulation in the product and the acceptability of the formulation.

Table.1. The formulations of healthy rice milk

Ingredient (%)	F1	F2	F3	F4	F5
Purple rice milk	100	70	70	70	70
Soy milk	0	10	8	10	12
Water caltrop milk	0	10	8	12	6
Sesame milk	0	10	14	8	12
Total	100	100	100	100	100

Physical-chemical analysis

The methods for analysis the chemical composition of sample (total protein, carbohydrate and fat) were followed the standard procedure recommended by AOAC (2000). Total of Anthocyanin content (TAC) was determined by the pH- differential method and visible in spectrophotometer with the difference in the absorbance of the pigments at 520nm and 700 nm. TSS was determined using a refractometer having a range of 0-32°Brix (Alla, France). Before measurement of TSS of sample, a refractometer calibrated using double distilled water. A drop of the rice milk will place on the sample slot of refractometer and the TSS of the sample will record and express in °Brix.

Optimizing enzymatic extraction of rice milk

A statistical experimental design based on Central Composite Design (CCD) was planned. The two independent variables were incubation concentration of enzyme (X1) and the duration of hydrolysis (X2). The code values of the independent variables were –α (lowest level), -1(lower level), 0 (middle level), 1 (higher level) and α (highest level). Correspondences between these coded and actual values are showed in Table 2. The experimental design consisted of 10 points including two replication of the central point and the result of total soluble solid was evaluated.

Table 2. Matrix of experimental central composite design (CCD) for enzymatic extraction of rice milk

Treatment (No.)	Concentration of enzyme	Time of hydrolysis
1	0	0
2	-1	-1
3	1	-1
4	-1	1
5	1	1
6	- α	0
7	+ α	0
8	0	- α
9	0	+ α
10	0	0

2.3 Sensory evaluation

The sensory properties of rice milk developed by nine member trained sensory evaluation panelists constituted from the faculty of Food Technology, Can tho University, Vietnam. CATA (Check All That Apply) questions and QDA (Quantitative Descriptive Analysis) training consists of evaluation rice milk by use of the descriptive terms developed to describe as quantify in aroma, flavor, texture and aftertaste characteristic of rice milk Attribution quantified and intensity rating scale from 0 to 10 (where 0=attribute not detected and 10= attribute extremely strong).

2.4 Statistical analysis

Analysis of variance (ANOVA) followed by Duncan's multiple range tests ($P<0.05$) was used to determine a significant difference between samples and make response surface curves and contour plots by STATGRAPHICS CENTURION XV.I software. For sensory evaluation, PCA was applied using XLSTAT (2007, Addinsoft, New York). PCA is a multivariate statistical method that entails data reconstruction and reduction. PCA generates a set of new orthogonal axes or variables known as principal components (PCs) from the original variables. The data sets presented on the orthogonal axes are uncorrelated with one another, and express much of the total variability in the data set through comparison of only a few PCs.

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3. RESULTS AND DISCUSSION

3.1 Effect of Enzymatic hydrolysis

Liquefaction process

The result of liquefaction is shown inTable3. The effects of α-amylase concentration and hydrolysis time on total soluble solid were measured in terms of Brix value for each process.

Table3. Effect of α-amylase concentration and liquefaction duration on total soluble solid content

α-amylase concentration (%)	Hydrolysis time (min)	Total soluble solid* (^{o}Brix)
0.05	35	9.6^{a}
	40	10^{ab}
	45	11^{c}
	50	11.33^{cd}
	55	12^{d}
	60	12^{d}
0.1	35	10.6667^{bc}
	40	11^{c}
	45	11.33^{cd}
	50	12^{c}
	55	12^{c}
	60	12^{c}
0.15	35	10^{ab}
	40	11^{c}
	45	11.13^{cd}
	50	11.4667^{cd}
	55	13^{d}
	60	13^{d}
0.2	35	10.6667^{cd}
	40	11c
	45	11.13^{cd}
	50	11.4667^{cd}
	55	13^{d}
	60	13^{d}

Note: (*) Mean of 3 replicates. Means in the same column followed by the same lowercase letters are not significant different at P>0.05

The results showed that total sugar increased in the first 35 min with the initial 5^{o}Brix and reach a peak highest to 13 in enzyme does at 0.15% with time 55 min and slowly decreased and constantly for the next run time. It indicated that α-amylase could convert the purple rice starch rapidly because of the high enzyme activity. Increasing total sugar concentration that showed in Brix degree was similar with increasing of reducing sugar concentration. Increasing of reducing sugar concentration indicated that the product was formed as a function of time and concentration of enzymes. As expected, the amount of product formed increased with time, although eventually the time was reached when there was no change in the concentration. In the various concentration of addition enzyme, the Brix value increased rapidly in 35 min then it rose up slowly until constant Brix value at 13%. It observed that the amylose and amylopectin running out at those concentrations becomes the product. For the concentration, 0.05% and 0.1% showed the maximum total sugar formed until Brix 12%. They were the lowest substrates concentration so the amylose and amylopectin which were converted to glucose were lowest than the other concentration of enzymes. Thuy *et al.* (2014) reported in the total soluble solid content of solution from the red rice hydrolysis gradually went up from 11.99% to 12.60% (wet basis) by the increase in α-amylase concentration between 0.05% and 0.20%. However, this content slightly decreased when the enzyme concentration surpassed 0.20%. Permanasari *et al.* (2018) also studies the effect of substrate and enzyme concentration on the glucose syrup production from red sorghum starch by enzymatic hydrolysis. The highest reducing sugar concentration was 191.60 g/l, 17.44% glucose observed for 40% substrate and 0.27% enzyme concentration and slowly decreased for the next period time. However, in term of increased the temperature to higher temperature were found α-amylase inactivation and decreased in the residual starch concentration in corn, rice and wheat starch.

Saccharification process

The result of saccharification is shown in Table 4. gluco-amylase activity in the first period of hydrolysis was not increasing TTS content almost treatments in the enzyme does between 0.05% to 0.1% with the duration of hydrolysis. However, the Brix value in a concentration of 0.15% at 40 min was increased slowly and became constant. It means that the total sugar production had almost reached the maximum value at the end of the liquefaction process for each concentration.

Table 4. The effect of gluco-amylase concentration and sacchrification duration on TTS

Gluco-amylase concentration (%)	Hydrolysis time (min)	Total soluble solid (*) (^{o}Brix)
0.05	35	12.33^{bcde}
	40	12.33^{bcde}
	45	12.33^{bcde}
	50	12.6667^{cdef}
	55	12^{abcd}
	60	12^{abcd}
0.1	35	12^{abcd}
	40	12.5^{cdef}
	45	12.5^{cdef}
	50	12.33^{bcde}
	55	11.33^{ab}
	60	11^{a}
0.15	35	12.16^{bcde}
	40	13.5^{f}
	45	12.83^{def}
	50	13^{def}
	55	11.66^{abc}
	60	11.33^{ab}
0.2	35	12.8^{def}
	40	12.8^{def}
	45	13.13^{ef}
	50	13^{def}
	55	12.6^{cdef}
	60	12.6^{cdef}

Note: (*) Mean of 3 replicates. Means in the same column followed by the same lower case letters are not significant different at P>0.05

The addition of gluco-amylase in the solution did not provide a significant improvement to the Brix value because starch hydrolysis was decreased may cause the inhibition of the enzyme by the addition of the solution. Also, the decrease in hydrolysis could be related to mass transfer limitation as the viscosity of the solution increased by the addition of glucose amylase. At low viscosity, starch and enzyme molecules move freely to contact each other; therefore, the reaction takes place. On the other hand, at high viscosity, the molecules cannot move freely because of viscous effects or reduced water activity; therefore, the rate of reaction decreases. The starch hydrolysis inhibition was also reported by Apar and Özbek (2005) in their work showed the decreasing of glucose on rice starch hydrolysis by adding the various amount of material. And also, the decrease in the rate of the mass transfer due to the addition of glucose may cause a lower hydrolysis degree.

Optimization of liquefaction process

The results of statistical analysis including the regression coefficient, P values for linear, quadratic and combined effects of the variables were given in Table 5. The larger the magnitude of the F-value and the smaller the P-value, indicate more significant of the corresponding coefficient and its effect on hydrolysis of purple rice starch by α-amylase. The P-values are used as a tool to check the significance of each of the coefficients and to understand the interactions between the best variables. Positive coefficients for X_1 (Enzyme concentration), X_2 (time) indicates a linear effect to increase on hydrolysis of rice starch by α-amylase. The quadratic effect of enzyme concentration and time had a significant effect ($p<0.05$). A good fit of a model, regression coefficient R^2 should be at least 80% and according to Zabeti *et al.* (2009). The correlation model obtained from the experiment satisfies the above condition was 0.878. The R^2 value implies that the sample variation of 87.8% for total soluble solid is attributed to the factors.

Modeling fitting

$$Y = 109.397 + 104.571X_1 - 4.3941X_2 - 350.0X_1^2 + 0.0390635X_2^2 \quad (1)$$

Where Y=Total soluble solid, X1= Concentration of enzyme (%) and X2 =time of hydrolysis (min)

Table 5.Analysis of Variance for total soluble solid in the liquefactions process

Source	Mean Square	F-Ratio	P-Value
X_1	4.3713	61.20	0.0000
X_2	6.36948	89.17	0.0000
X_1^2	0.656241	9.19	0.0064
X_1X_2	0.0	0.00	1.0000
X_2^2	0.33483	4.69	0.0420
Lack-of-fit	0.0759829	1.06	0.3856
Pure error	0.0714286		
R-squared	0.878099		
Adj R-squared	0.852703		

The response surface plot showed the interaction effect of enzyme does and hydrolysis time on the total soluble solid as shown in "Figure1".It can be seen that the degree of Brix decreased slowly when using a high concentration of enzyme to 0.2% and increased hydrolysis time. However, the Brix degree did not affect when the time increased above 60 min. It was observed that at the middle level of enzyme concentration (0.1 to 0.15% (w/v)) and at the middle level of hydrolysis time (55 to 57 min),the total soluble solid contents was high (14%). Based on the Response Surface Methodology (RSM) using the central composite design (CCD) model the optimal values of enzyme concentration and duration in the liquefaction process by α-amylase was estimated in actual units were 0.149% and 57.8284 min, respectively. From the optimization results Table 6 showed that concentration of α-amylase 0.149% and time of hydrolysis 57.8284 for the highest Brix content (14.2499%). Calculated from the model was performed no significant difference between the optimum and experimental value.

Table 6. Testing the optimum value obtained from the model compared to the experiment

	Concentration of enzyme (α-amylase)	Time of hydrolysis	^{o} Brix
Model	0.149	57.83	14.2499
Experimental	0.149	58	14±0.58

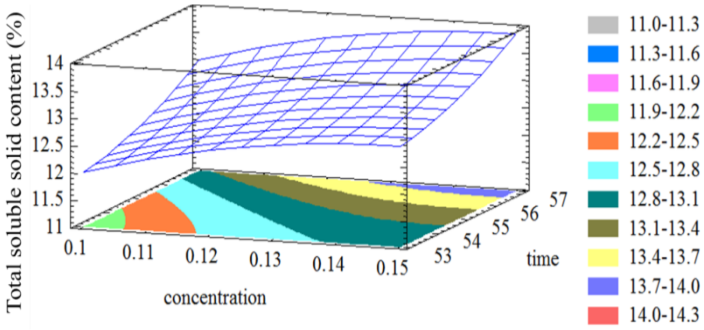

Figure.1 Response surface plot of the effect of enzyme concentration (α-amylase) and hydrolysis time on total soluble solid contents.

Optimization of saccharification process

The dependent variable values were coded and the response variable was the total soluble solids. Both enzyme concentration and time variables, as well as their interaction, were significant at $P<0.05$. As observed in the table, both variables were significant, with a higher effect from the concentration of enzyme. Both variables enzyme dose and time effects were positive. Concerning the interaction, the effect was negative. The mathematical model derived from the statistical analysis is presented in (Equation 2), which gives R^2=0.9667 the model is a good fit as represented by the high value of the coefficient of determination (R^2). For the experimental data, where Y represents the number of total soluble solid, X1 represents the numerical value of the ratio of the enzyme (%) and X2 represents the numerical value of the hydrolysis time (min). the effect of enzyme concentration and time on hydrolysis described in the form (Equation 2) a second-order polynomial model in code units. The response surface plot “Figure 2” described the interaction effect between pair of factors on hydrolysis purple rice starch in saccharification process using glucose amylase. It was observed that at the middle of level enzyme concentration (0.125 to 0.175 (w/v)) and at the middle of time (38 to 42 min) the degree of Brix was high 16%. The statistical from Response Surface Methodology (RSM) using the central composite design (CCD) model the optimal values of enzyme concentration and duration in saccharification process by gluco-amylase was estimated in actual units were 0.143% and 40.94 min, respectively. From the optimization results showed that concentration of gluco-amylase 0.143% and time of hydrolysis 40.94 min for the highest Brix content (14.2499%). Calculated from the model was performed no significant difference between the optimum and experimental value given in Table7.

Table 7. Testing the optimum value obtained from the model compared to the experiment

	Concentration of enzyme (gluco-amylase)	Time of hydrolysis	°Brix
Model	0.143	40.94	16.4432
Experimental	0.143	41	16.13±0.23

Model fitting:

$$Y = -484.959 + 996.521X_1 + 21.0077X_2 - 2050.02X_1^2 - 10.0X_1X_2 - 0.239064X_2^2 \quad (2)$$

Table 8. Variance analysis to determine the optimal of enzyme does and time on saccharifaction process

Source	Mean Square	F-Ratio	P-Value
X_1	1.70853	12.57	0.0239
X_2	4.68383	34.45	0.0042
X_1^2	7.50443	55.19	0.0018
X_1X_2	1.0	7.35	0.0534
X_2^2	4.18013	30.74	0.0052
Lack-of-fit	0.154633	1.93	0.4690
Pure error	0.0714286		
R-squared	0.966773		
Adj R-squared	0.925238		

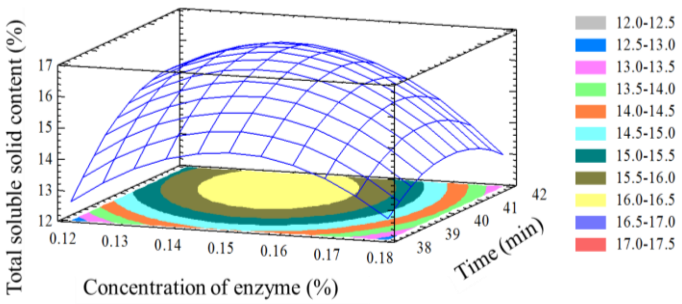

Figure.2 Response surface plot of the effect of enzyme concentration (gluco-amylase) and hydrolysis time on total soluble solid content

3.2 Effect of mixing various ratio of soybean, water caltrop or sesame with rice milk on the chemical compositions and sensory attributes

Chemical properties

The summary of chemical composition of five formulations of rice milk is given in Table 9. These results showed that significant differences existed ($p<0.05$) in protein, carbohydrate, lipid and anthocyanin of all formulations.All the analyses were conducted in triplicate and mean±standard deviations.

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Table 9. Chemical properties of formulations

Formulation	Properties			
	Protein (%)	Carbohydrate (%)	Lipid (%)	Anthocyanin (mg/l)
F1	1.08±0.025[a]	12.09±0.01[d]	1.10±0.04[c]	1.50±0.14[a]
F2	1.04±0.02[a]	6.24±0.01[a]	0.72±0.15[b]	0.70±0.10[a]
F3	1.02±0.27[a]	8.61±0.01[c]	1.23±0.03[c]	1.02±0.4[a]
F4	1.85±0.34[b]	7.30±0.01[ab]	1.32±0.06[c]	2.89±0.01[b]
F5	1.07±0.22[a]	8.07±0.0[bc]	0.19±0.08[a]	1.58±0.02[a]

Note: Means with the different letter in the same raw are significantly different ($p<0.05$)

The protein content of purple rice milk were ranged from 1.02 to 1.85% as adding mixture as soy milk, sesame milk and water caltrop milk increased the protein contents significantly ($p<0.05$). It observed that adding other materials in purple rice milk able enhanced the protein content in the products according to the result the treatment contenting 100% of purple rice milk had the lowest content of protein while the highest content of protein was recorded in the formula 4. The results of this study were in agreement with Atallah and Barakat (2017) who showed that protein content increased with increasing the amount of soybean milk. Supavadee and Phakhwan (2012) reported the production of rice milk from young rice and cereal beverage when mixtures with sesame were increased the content of protein from 0.06% to 0.93%.

Lipid content of control and different combinations of purple rice milk samples revealed a significant difference ($p<0.05$) among treatments. Fat content of sample was ranged from 0.19 to 1.32%. The fat content of rice milk increased when the sample mixture with other materials and also the highest content of lipid belonged to formula 4 while the lowest one was formula 5. This may due to the rice milk produced from rice had fewer amounts of fat content and also water caltrop as mixture in the purple rice milk has not a significant source of lipid. According to the report from Chiang *et al.* (2007) the lipid contents in the kernels of T. *quadrispinosa* and T.*taiwannensis* were 0.5-0.6% (dry basis)

The carbohydrate content of purple rice milk-based mixture with other materials varied from 6.24-12.09%. The effect of purple rice milk substitution with other materials was significant ($p<0.05$). when mixture rice milk with other materials, a significant difference was observed in the total carbohydrate content because the treatment content 100% of rice milk (formula 1) had the highest amount of carbohydrate while other formulas were decreased the carbohydrate content due to in rice has a large amount of starch are mainly amylose and amylopectin so when produced 100% of rice milk without mixing with others may had higher on the total carbohydrate. According to Perezgonzalez (2008), the average chemical compound of rice milk was made from rice and water has high in carbohydrate and sugar but low in protein, fat and saturated fat. Similarly, Belewu (2016) described the highest carbohydrate content for coconut milk when combined with rice milk.

Moreover, the bioactive compound as anthocyanin was contained in rice milk because this product was produced from the purple rice as well-known containing the anthocyanin compound. There were ranges from 0.7-2.89 mg/L. The effect of mixing various materials had significant ($p<0.05$). The highest content of anthocyanin belonged to the formula 4 accounting for 2.89 mg/l.

Generally, the chemical components of rice milk found in this study were near to those obtained by El TahirLimia (2015) who reported that the fat, protein, ash and carbohydrate content of rice milk were 0.18, 1.87, 0.42 and 5.40%, respectively. Ismail (2016) reported the amount of rice milk components in his study which were 12.30% of total solid, 0.3% of fat, 1.62% of total protein and 0.39% of ash. The rice milk is low in protein, fat, fiber and micronutrient, but high in carbohydrate. In particular, rice milk is consumed by people with lactose intolerance to avoid hormones in animal milk due to cancer- related illness, allergy, a macrobiotic diet or vegetarian

Sensory evaluation

All of the formulations were significantly different in cooked aroma, viscosity, chalky and lip, and mouthfeel. In the PCA result sensory map of samples, PC was carried out on CATA question. The total variable of PCA accounted for 94.92% while the first dimension explains 85.27% of the variance in the data set and second dimension explains an addition 9.66% of the variance. As shown in "Figure 3a", the first dimension associated with the term caramelized, sweet, chalky and malty and correlated negatively with the terms of bitter, viscosity, cooked aroma, caramelized aroma and salty. According to cluster analysis samples categorize into four groups. One group located at positive value of the first dimension that comprise formula 4 and was explained with terms of caramelized, sweet and chalky. Formula 3 was placed at a negative value of the first dimension and was specified by the term of malty. Formula 2 and formula 5 were placed at the positive value of the second dimension and were specified by the terms bitter, lip and mouth feeling and lack of freshness. Finally, sample formula 1 was situated apart from the rest and mainly characterized by the terms cooked, viscosity, bitter, caramelized aroma, cooked aroma and salty. In addition, to evaluate each attribute, panelists were asked to evaluate the overall liking of five formulas of purple rice milk. It was obviously found that in term chalky, caramelized and sweetness correlated to overall liking. The trend of liking showed that formula 4 received excellent appreciation by the panelists "Figure 3b". The sample had a low amount of fat and content chalky attribute because this milk were produced from the grain and plants which have a high content of starch but panelists perceived the most preference of sample.

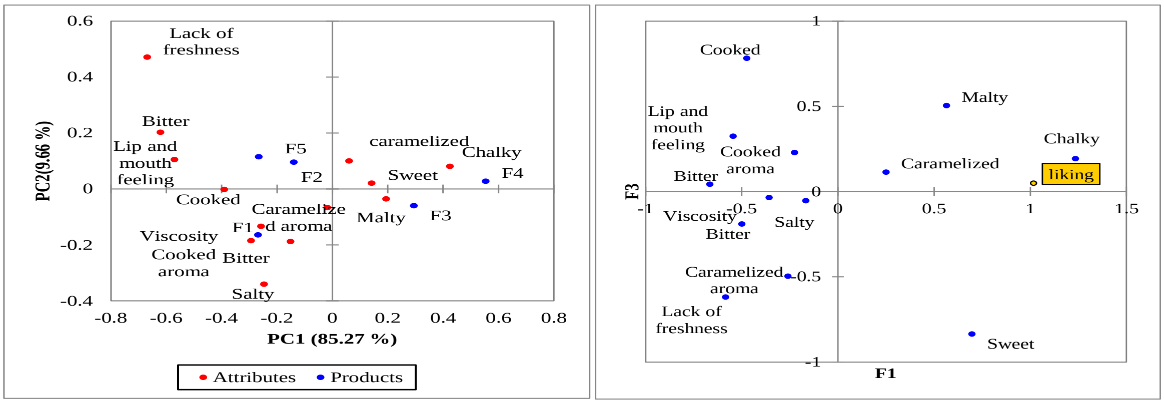

Figure 3."(a)"The bi-plot graph explains the relationship between the purple rice milk samples and all sensory attributes and "(b)" The overall quality rating of samples

From the results of QDA, each formula corresponds to the mean value attribute by the panelist group. Samples are located near the descriptors that their characteristics "Figure 4". In the present study, the two principal components were used together and explained 85.63% of the total variability observed among the treatments. The rice milk formula 4 was characterized by descriptor chalky and overall quality rating was had the same term with CATA question. Formula 5 and formula 2 were characterized by bitter taste, lip and mouth feeling aftertaste, sweet and viscosity. Formula 1 was presented primarily by caramelized tastes, caramelized aroma, cooked taste, bitter and malty. Formula 3 was an exception for all sensory descriptors. The result of this study was in agreement with Thuy *et al.* (2015) examined the sensory attributes of the rice milk products prepared with a combination of added cream milk and total soluble solid content. The principal component analysis identified two significant principal components that accounted for 89.86% of the variance in the sensory attribute data. The important sensory attributes of rice milk corresponded to sweetness, fatty taste, rice flavor (chalky), cow milk flavor, milk skin, sedimentation, and brown color.

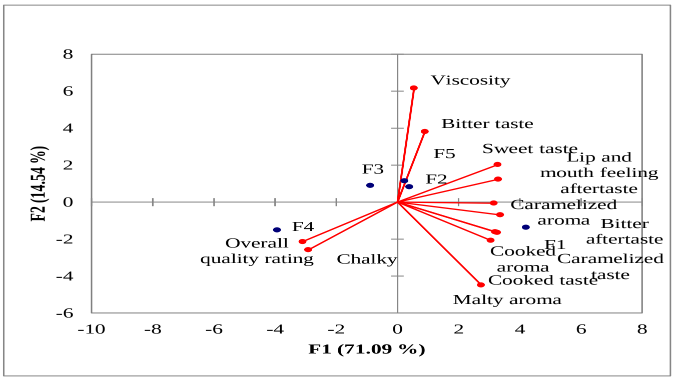

Figure 4. Principle component analyses (PCA) of sensory data of milk produce. PCA loadings and scores for principle component (PC1 and PC2) including all evaluated sensory descriptors.

4. CONCLUSION

The optimal conditions for extraction of purple rice milk using α-amylase were 0.149 % (v/w) of enzymes concentration and 57 min of hydrolysis. In the saccharification process by gluco-amylase was using 0.143% of enzyme does and 40.94 min of hydrolysis. Under the optimum condition, the rice milk increased total soluble solid as 14% and 16%, respectively. Concerning to the term of enhancing the nutrition of rice milk, it is clear that the formula 4 with 70% rice milk, 10% soybean milk, 12% water caltrop milk and 8% sesame milk was presented better sensory quality than other formulas with high nutrients content.

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