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Quality performance of Indonesian frozen dessert *(es puter)* enriched with black glutinous rice (*Oryza sativa glutinosa* L.) and green tea (*Camellia sinensis* L.)

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Abstract

Fermented black glutinous rice (*Oryza sativa glutinosa* L.) and green tea (*Camellia sinensis* L.) contain bioactive compounds including anthocyanins and flavonoids which have potential to enrich *es puter*, one of the favorite traditional frozen desserts in Indonesia, as a functional food. This study aimed to get the best formula of *es puter* enriched by fermented black glutinous rice and green tea. The local variety of fermented black glutinous rice (FBGR) was prepared by steaming until cooked then fermented by using ragi tape (traditional yeast cake) at 27°C for 3 days. A factorial design experiment was used to study the effects of proportion of FBGR (25, 30, and 35%) and green tea powder (2, 3, and 4%) on the physical and chemical properties of *es puter*. The results showed that the FBGR had higher total phenolic content, antioxidant activity, and anthocyanin content than the unfermented black glutinous rice ($p<0.05$). The best *es puter* formula consisted of 30% FBGR, 4% green tea powder, 51.2% coconut milk, 14% sugar, 0.4% salt, 0.2% sodium carboxymethyl cellulose and 0.2% glycerol monostearate. The physical characteristics of this formula were accepted with 33.46±0.26% total solid, 7.31±0.10% overrun, 2,794.33±0.58 cP viscosity, and 9.57±0.87 min melting time. The antioxidant activity (DPPH), phenolic content and anthocyanin content were 1,865.81±161.63 µmol TE/100g dry basis, 0.09±0.01 GAE in g/100g sample and 0.30±0.11 mg/100g sample dry basis, respectively. This formula had overall liking score 6.5 which indicated that the modified *es puter* was liked moderately. In addition, consumer acceptance was conducted using 100 consumers and sixty-five percent of consumers accepted this product.

Introduction

Es puter is one of the favorite traditional frozen desserts in Indonesia. The physical appearance of *es puter* product is just like an ice cream, but its taste is more like to ice milk or mellorine, due to its nature of coarser ice crystals and lower fat content. It contains coconut milk as the main ingredient. *Es puter* is much liked by Indonesian people because it is tasty and has affordable price. It is consumed in very wide age range, from children to adults. *Es puter* has a potential to be developed in Thailand as coconut production is widely grown, yielding about 1,055,320 ton in 2011 (FAOSTAT, 2011). *Es puter* can be modified by adding other functional ingredients in order to increase its healthy benefit.

This research focused on, firstly, utilization of functional compound such as flavonoids and anthocyanins of the black glutinous rice which is commonly grown in Thailand. Vichapong *et al*. (2010) revealed that varieties of Thai pigmented rice such as black glutinous rice has higher phenolic compound, total flavonoid and antioxidant activity than the normal white staple rice. In addition, the anthocyanin content of black glutinous rice is higher than red rice (Sung Yod, Mon Poo and Hom Mali Daeng) and purple rice (Hom Nil and Riceberry) (Plaitho *et al*., 2012). Anthocyanins are a group of pigments commonly found in pigmented rice such as purple, black and red rice. These compounds have biological property as scavenging free radicals (Wang and Jiao, 2000). Moreover, the fermented black glutinous rice has higher phenolic content, anthocyanin, antioxidant activities, and antimutagenicity (Plaitho *et al*., 2012). Secondly, this research also aimed to study the use of ingredients rich in antioxidant in the product. The active constituents in green tea are powerful antioxidants called polyphenols. Tea is reported as containing nearly 4000 bioactive compounds of which one third are contributed by polyphenols (Tariq *et al*., 2010). Flavonoids (and their fraction, catechins) are the basic phenolic compounds in green tea and are responsible for antioxidant activities like neutralization of free radicals that are

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Table 1. Formulation of *es puter*

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
	Concentration* (%)								
CMC	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
GMS	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Sugar	14	14	14	14	14	14	14	14	14
Fermented black glutinous rice	25	25	25	30	30	30	35	35	35
Green tea powder	2	3	4	2	3	4	2	3	4

*from the amount of coconut milk that added

formed in the process of metabolism (Horzie *et al*., 2009). The USDA (2003) published a database for the flavonoid content of selected foods. It showed that the main flavonoids available in green tea are catechins (flavan-3-ols) which are; epigallocatechin-3-gallate (EGCG), that represents approximately 59% of the total of catechins; epigallocatechin (EGC) (19% approximately); epicatechin-3-gallate (ECG) (13.6% approximately); and epicatechin (EC) (6.4% approximately) (McKay and Blumberg, 2002). Wolfram *et al.* (2006) report evidence that catechins and epigallocatechin gallate (EGCG) in green tea reduce adipocytes differentiation and proliferation, lipogensis i.e., birth of new fat cells; fat mass, body weight, fat oxidation, plasma levels of triglyceride, free fatty acids, cholesterol, glucose, insulin and leptin and increased beta-oxidation and thermogenesis. Chu *et al*. (2010) reported that green tea "catechins" are among a number of antioxidants such as vitamin C, vitamin E, lutein, and zeaxanthin thought capable of protecting the eye. Combination of these two products, fermented glutinous rice and green tea, as enricher of the *es puter* is expected to increase popularity and health benefits of *es puter*. The objective of this study was to get the best formula of *es puter* enriched by fermented black glutinous rice and green tea.

Materials and Methods

Materials

Black glutinous rice was obtained from local market in Chiang Rai, Thailand. Coconut milk (AROY-D, Thailand), salt (Aro, Thailand), sugar (Mitr Polt, Thailand) and green tea powder (Suwirun, Thailand) were also received from local market. A commercial yeast cake starter called "ragi-tape" (Cap Bawang) was bought from local market in Jakarta, Indonesia. Sodium carboxymethyl cellulose and glycerol monostearate were obtained from Sigma-Aldrich, St. Louis, Mo, USA.

Chemical reagents used in this research were Folin-Ciocalteu's phenol reagent and H2O2 (Merck, Darmstadt, Germany), anhydrous sodium carbonate and methanol (Ajax, Amsterdam, Netherlands), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and trolox-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Sigma-Aldrich, St. Louis, Mo, USA), potassium chloride buffer pH 1, sodium acetat buffer pH 4.5, hexane, and distilled water. All chemicals and reagents were of analytical grade.

Fermented black glutinous rice preparation

Black glutinous rice was soaked in water for 12 h, and then steamed at 98°C for 2 h. The sample was cooled and then mixed with "ragi-tape" that previously had been crushed into powder (0.27%w/w of sample) and fermented in plastic bag at room temperature (±27°C) for 3 days.

Es puter preparation

A factorial design was used in this research to study the effect of fermented black glutinous rice (FBGR) (25, 30, and 35%) and green tea powder (2, 3, and 4%) proportions to fixed formula of *es puter* (Table 1). The *es puter* formulations had this following composition: coconut milk as the basis (8% fat), 0.2% sodium carboxymethyl cellulose (CMC), 0.2% glycerol monostearate (GMS), 0.4% salt, 14% sugar, 25% - 35% fermented black glutinous rice, and 2% - 4% green tea powder.

Physical quality determination

Overrun

Overrun was determined according to Marshall *et al*. (2003) by using a standard 100 ml cup. *Es puter* mix and frozen *es puter* at 100 ml were weighted and overrun was calculated according to the equation:

$$\text{Overrun (\%)} = \frac{W1 - W2}{W1} \times 100$$

Where: W1= weight of the *es puter* mix (g) and W2= weight of the frozen *es puter* (g)

Viscosity

Viscosity measurement was carried out according to Nadeem *et al.* (2009) by placing *es puter* mix (180 mL) in a cylinder glass (6 cm diameter × 9 cm height). After aging the mix at 4°C for about 24 hours then measuring viscosity with a Brookfield viscometer (Model RVDVII, Brookfield Engineering Laboratories, Inc., MA, USA). The viscometer was operated at 20 rpm (spindle number 2).

Melting time

Melting time was determined based on the time required for sample to melt at room temperature (±27^{o}C) by taking a scoop sample (5.00 g) and then placing it on a plate (Clark *et al*., 2009).

Chemical analysis

Sample preparation

Sample was extracted by method of Sutharut and Sudarat (2012). Briefly, one gram of each sample was transferred into a test tube (25 × 150 mm), methanol (3 mL) was added, and the mixture was mixed using a vortex for 30 sec. The test tubes were then capped and placed in a 60°C water bath for 20 min. The test tubes were mixed again by vortex twice during the incubation. Then, the methanol layer in each tube was separated by centrifugation at 10,000 rpm for 10 min. The solvent supernatant was transferred to a 10 mL volumetric flask. The residue was again mixed with 3 mL of methanol. The supernatant was adjusted to 10 mL and kept at 0°C until analysis.

DDPH radical scavenging activity

Diluted sample extract (50 µL) was mixed with of 60 µM DPPH solutions (1950 µL). then kept in the dark for 30 minutes after which the absorbance was measured at 517 nm using methanol as blank and Trolox as standard. The radical scavenging activity was presented as µmol Trolox/100g dry basis (Molyneux, 2004).

Total phenolic content

The total phenolic content (TPC) was determined using gallic acid as a standard. Diluted sample extract (1.0 mL) was transferred into a tube containing 10% v/v Folin-Ciocalteu's reagent (5.0 ml). Then, 7.5% w/v sodium carbonate solution (4.0 mL) was added. The mixture was kept at room temperature for 1 h and then the absorbance was measured at 765 nm using water as a blank. The TPC was expressed as gallic acid equivalents (GAE) in g/100g sample (ISO 14502-1, 2005).

Total anthocyanin content

The total anthocyanin content (TAC) was determined using the pH-differential method. Briefly, extracted solution (1 mL) was transferred into a 10 mL volumetric flask. Two dilutions of the sample was prepared by one sample was adjusted for its volume with potassium chloride buffer (pH 1.0), and the other was adjusted with sodium acetate buffer (pH 4.5). Let these dilutions equilibrate for 15 min. Measure the absorbance of each dilution at the 510 and 700 nm, against a water blank (Guisti and Wrolstad, 2001). All measurements should be made between 15 min and 1 hr after sample preparation. The absorbance of the diluted sample (A) was calculated as follows:

$$A = (A_{510} - A_{700})\ pH_{1.0} - (A_{510} - A_{700})\ pH_{4.5}$$

The monomeric anthocyanin pigment concentration in the original sample was calculated by using the following formula:

Monomeric anthocyanin pigment (mg/L) =

$$\frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)}$$

and it was converted to mg of total anthocyanin content /100 g sample.

Where MW is the molecular weight, DF is the dilution factor, and ε is the molar absorptivity. The pigment content is calculated as cyanidin-3-glucoside, where MW = 449.2 and ε= 26,900

Proximate analysis

Samples were analyzed for total solids and moisture content (AOAC 941.08, 2007), ash content (AOAC 923.03, 2007), protein content by micro Kjeldahl (AOAC 960.52, 2007), lipid content by soxhlet extraction (AOAC 963.15, 2007) and carbohydrate content by difference method.

Sensory evaluation

Sensory analysis was carried out to determine the best sample using thirty five untrained panelists (Lawless and Heymann, 1998).Three formulations were given to the panelists in frozen condition. Then, 9-point hedonic scale was used for evaluating the *es puter* on the basis of acceptance of appearance, color, aroma, bitterness, sourness, taste, texture, and overall liking.

Consumer test

Consumer test was done to determine the acceptance of *es puter* final product. The location of consumer test was in the central location of Mae Fah Luang University, Thailand, with 100 responses (Meilgaard *et al*., 2007).

Table 2. Overrun, viscosity, melting time, and total solid of *es puter*

Formulations		Overrun (%)	Viscosity (cP)	Melting Time (min)	Total Solids (%)
Green tea powder	Fermented black glutinous rice				
2%	25%	24.77±0.79[a]	476.93±0.90[i]	6.01±0.29[i]	31.60±0.18[h]
	30%	20.07±0.33[a]	952.60±0.53[h]	7.57±0.68[fic]	32.32±0.18[g]
	35%	18.98±0.66[b]	1,119.00±0.89[g]	8.56±1.16[c]	32.47±0.18[g]
3%	25%	17.59±0.31[d]	1,226.47±0.47[f]	6.68±1.03[ig]	31.89±0.46[eh]
	30%	15.45±0.71[e]	1,400.67±1.53[e]	8.86±0.74[dfb]	32.53±0.76[degb]
	35%	12.30±0.37[f]	1,916.67±0.49[d]	10.32±0.59[b]	33.40±0.07[d]
4%	25%	9.95±0.82[g]	2,247.33±0.75[c]	7.68±0.55[g]	32.95±0.08[b]
	30%	7.31±0.10[h]	2,794.33±0.58[b]	9.57±0.87[d]	33.46±0.26[b]
	35%	5.35±0.41[i]	3,523.00±1.00[a]	12.93±0.27[a]	34.22±0.41[a]

Results were expressed as mean±SD (n=3)
Data with different letters in column indicated significant differences ($p<0.05$)

Statistical analysis

All data was used for one-way analysis of variance to identify different among means by t-test using SPSS program (version 17.0) at 95% confidence level.

Results and Discussion

Overrun

Overrun is defined as the volume of air whipped into the product during the freezing process due to the air trapped by short-chain protein, fat, and lactose (Arbuckle *et al.* 1986). Commercial *es puter* has overrun values around 4.37-7.54% (Setiawan, 2009) however overrun values in this study ranged between 5.35-24.77% (Table 2). *Es puter* in this study had higher overrun values than commercial *es puter*. *Es puter* contains relatively low protein and fat, moreover it does not contain lactose from milk. The presence of GMS as emulsifiers in *es puter* improved the ability of the mix to incorporate air (Kilara and Chandan, 2007) while commercial *es puter* does not contain it. Overrun of 4% green tea:30% FBGR (7.31±0.10%) and 4% green tea:35% FBGR formulations (5.35±0.41%) approached the range of commercial *es puter* overrun values (4.37-7.54%). Table 2 shows that the higher the content of green tea powder and FBGR the lower of the overrun value. Increase in the green tea powder content increased the viscosity of the *es puter* mix. Arbuckle (1986) reported that increasing the viscosity will increase the surface tension which increases the capability to entrap air. However, our data shows that the higher the *es puter* viscosity, the lower was the overrun which was caused by adding the intact glutinous rice into the mix after the homogenization process. In the homogenizer, the large fat droplets are elongated and broken up into a fine emulsion of much smaller droplets (about 1µm in diameter), greatly increasing the surface area. After homogenization, protein readily adsorbs to the expored surface of the fat droplets. The proteins are mostly adsorbed on the aqueous side of fat-matrix interface, with hydrophobic parts at the interface. Interaction between the proteins on the outside of the droplets make it harder for the droplets to come into close contact which is known as steric stabilization (Clarke, 2008). However, the stabilization that had been formed was disturbed by the presence of fermented black glutinous rice and made the mix was difficult to incorporate and stabilize air bubbles when the mix was frozen. It caused the overrun value to decrease with increasing amount of FBGR.

Viscosity

Viscosity is a measurement of resistance to flow of a fluid (Toledo, 2007). A certain level of viscosity seems essential for proper whipping and for retention of air (Marshall and Arbuckle, 2000). The viscosity of an *es puter* mix is affected by compositions (fat and stabilizer), type and quality of ingredients, processing and handling of the mix (pasteurization, homogenization, and aging), concentration (TS content), and temperature (Marshall and Arbuckle, 2000). Commercial *es puter* contains some ingredients as filler such as hunkue flour and tapioca starch (Hestiana, 2009). The presence of starch in the commercial *es puter* plays a role in increasing its viscosity. However, there were two factors that play a role in increasing the viscosity of *es puter* in this study that were stabilizer and total solid. One of the stabilizer's functions is to develop viscosity in the *es puter* mix, since a more viscous mix has a better

Table 3. Determination of three selected es puter formulations based on overrun, viscosity, and melting time that approach range of physical quality of commercial *es puter*

Parameter	Green tea powder (%)	2			3			4		
	Fermented black glutinous rice (%)	25	30	35	25	30	35	25	30	35
Overrun		-	-	-	-	-	-	-	√	√
Viscosity		-	-	-	-	-	-	√	√	√
Melting time		√	√	√	√	√	-	√	-	-
Total		1	1	1	1	1	0	2	2	2

– : formulation of *es puter* that did not approach range of physical quality of commercial *es puter.*
√ : formulation of *es puter* that approached range of physical quality of commercial *es puter*

capacity to retain air bubbles (Cottrell *et al*., 1980; Bolliger *et al*., 2000a; Chavez-Montes *et al*., 2004). Green tea powder added to *es puter* increased its total solid replacing the water content in the mix which also happened to increase the viscosity and nutritive value, and improve the body and texture of the *es puter* (Marshall and Arbuckle, 2000). The data in Table 2 shows that the viscosity values in this study (476.93-3,523.00 cP) were lower than commercial *es puter* (4,000-6,000 cP). It shows that starch gave a stronger effect on viscosity of *es puter* mix than stabilizer and green tea powder. Table 2 also shows that the higher the content of green tea powder and FBGR, the higher the product's viscosity. The highest viscosity was the formulation with 4% green tea powder and 35% FBGR (3,523.00±1.00 cP). In addition, viscosity of formulations 4%:25%, 4%:30% and 4%:35% were in the same range of commercial *es puter*'s viscosity (Setiawan, 2009).

Melting time

Melting time is the time required for *es puter* to perfectly melt at room temperature. Table 2 shows that *es puter* in this study had melting time (6.01-12.93 min) slightly higher than commercial *es puter* (6.64-9.08 min; Setiawan, 2009) and the longest melting time was formulation with 4% green tea powder and 35% FBGR. The meltdown characteristics are influenced by the protein stability, fat agglomeration, and air cell size. A partially coalesced three-dimensional network formed by the fat globules with air and ice is in part responsible for the melt resistance and smoother texture of the frozen dessert. Presence of surface-active proteins will stabilize the weak fat-serum interface first. Increased emulsification results in depletion of protein from the fat molecule increases fat destabilization, decreases melting rate and enhances shape retention during the melting process (Bolliger *et al*., 2000b). The factor that play a role in decreasing melting time of *es puter* in this study is stabilizer. In addition to producing smoothness in texture during eating, stabilizer can reduce the rate of meltdown (i.e. the rate at which the *es puter* loses mass as it melts) (Clarke, 2008) yet the commercial *es puter* does not contain it. The data showed the higher content of green tea powder and FBGR, the longer the product's melting time. Generally, as the viscosity increases, the resistance to melting increases too (Marshall *et al*., 2003). From melting time data, formulation 2%:25% (6.01±0.29 min), 2%:30% (7.57±0.68 min), 2%:35% (8.56±1.16 min), 3%:25% (6.68±1.03 min), 3%:30% (8.86±0.74 min), and 4%:25% (7.68±0.55 min) approached range of melting time of commercial *es puter* (6.64-9.08 min).

Total solids

The total solids is the sum of all ingredients other than water (Clarke, 2008). Products such as *es puter*s, sorbets, milk ices or water ices each contain a subset of the ingredients of ice cream. In the *es puter* in this study, total solids come from coconut milk, sugar, salt, stabilizer (CMC), emulsifier (GMS), green tea powder, and FBGR. Total solids replaced water in the mix, thereby increasing the nutritive value and viscosity and improving the body and texture of the ice cream (Marshall and Arbuckle, 2000). The data showed that total solids values of *es puter* were in the range 31.60-34.22% (Table 2). Total solids values of *es puter* in this study were higher than these in commercial *es puter* (19.47-23.08%). This was caused by adding FBGR. From Table 3, three selected formulations (4%:25%, 4%:30%, and 4%:35%) were shown by their overrun, viscosity, and melting time to approach the range of physical quality of commercial *es puter* (Setiawan, 2009). These three formulations were then analyzed further by chemical analysis and sensory evaluation to get the best formula.

Table 4. Antioxidant activity (DPPH), phenolic content, and anthocyanin content of *es puter* and unfermented and fermented black glutinous rice

Formulations *es puter*		Antioxidant activity (DPPH) (µmol TE/100g dry basis)	Phenolic content (GAE in g/100 g sample)	Anthocyanin content (mg of TAC/ 100g sample)
Green tea powder	Fermented black glutinous rice			
4%	25%	1,105.36±117.84[b]	0.08±0.01[ns]	0.28±0.02[ns]
	30%	1,865.81±161.63[a]	0.09±0.01[ns]	0.30±0.11[ns]
	35%	1,971.11±118.49[a]	0.08±0.01[ns]	0.32±0.10[ns]
Black glutinous rice	Unfermented	65.44±15.45[b]	0.04±0.01[b]	0.24±0.01[b]
	Fermented	248.42±9.90[a]	0.07±0.01[a]	0.36±0.07[a]

Results were expressed as mean±SD (n=3)
[ns]superscript in column was not significantly different (p>0.05)
Data with different letters in column indicated significant differences (p<0.05)

Antioxidant activity (DPPH)

Table 4 shows that antioxidant activity of FBGR was higher than unfermented black glutinous rice ($p<0.05$). The results of fermentation are consistent with other reports. Sadabpod *et al.* (2010) reported that DPPH scavenging activity of FBGR was higher than those of raw rice and cooked rice while fermented maize (Daker *et al*., 2008) and Phellinus-fermented adlay and rice (Liang *et al*., 2009) had better DPPH scavenging effect than that of unfermented material. Watermelon rind fermented by Saccharomyces cerevisiae was shown to have higher DPPH scavenging capacity than unfermented rind (Erukainur*e et al*., 2011). The increase in the DPPH scavenging activity of fermented rice might be due to the increase in phenolic and anthocyanin contents. Anthocyanins are natural phenolic pigments that were reported to scavenge free radicals such as superoxide (O_2-), singlet oxygen ($^{\cdot}O_2$), peroxide (ROO-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH-) (Wang and Jiao, 2000). Normally, the antioxidant activity of the anthocyanidins (aglycons) was generally greater than those of the corresponding anthocyanins (glycosides) (Wang and Stoner, 2008).

Table 4 also shows that antioxidant activity of 4%:30% and 4%:35% formulations was higher (1,865.81±161.63 and 1,971.11±118.49 µmol Trolox equivalent/100g dry weight, respectively) than formulation 4%:25% (1,105.36±117.84 µmol Trolox equivalent/100g dry weight) ($p<0.05$) while the phenolic and anthocyanin contents were not significantly different ($p>0.05$). The addition of fermented black glutinous rice played a role in decreasing the pH of *es puter*. Anthocyanins are more stable in acidic media at low pH values than in alkaline solutions with high pH values (Rein, 2005). Therefore, the higher the content of FBGR, the higher its antioxidant activity. In addition, Table 4 shows that antioxidant activity was not always linearly related with the phenolic compound content. Black glutinous rice contains bioactive compounds other than phenolic compounds such as phytic acid, γ–oryzanols, α–tocopherol, and γ–tocopherol (Moongngarm *et al*., 2012). These compounds contribute in increasing antioxidant activity of black glutinous rice. Antioxidant activity of *es puter* containing of green tea and FBGR compared with fermented black glutinous rice had higher antioxidant activity. The addition of green tea played a role in increasing antioxidant activity of *es puter*. Major catechins present in green tea, i.e. epicatechin (EC), epigallocatechin gallate (EGCG), epigallocatechins (EGC) and epicatechin gallate (ECG), have strong antioxidant potentials (Rai *et al*., 2012). Antioxidant activity of EGCG is about 25-100 times more potent than vitamin C and E and is the single most studied catechins in relation to health contributing potential (Cabrera *et al*., 2006).

Phenolic content

The phenolic content of FBGR (0.07±0.01 GAE in g/100 g sample) was higher than unfermented black glutinous rice (0.04±0.01 GAE in g/100 g sample) ($p<0.05$) (Table 4). The increase found in phenolic content of black glutinous rice after fermentation was similar to that found by Liang *et al.* (2009) who showed that Phellinus-fermented rice exhibited higher phenolic content than unfermented rice. Sadabpod *et al.* (2010) also reported that phenolic contents of fermented black glutinous rice were higher than those of raw rice and cooked rice. The greater phenolic content of the fermented black glutinous rice may be due to the enzymatic activities of starter organisms in ragi tape such as *Amylomyces*

Table 5. Hedonic result of three selected formulations of *es puter* and preference test result of the best formula of *es puter*

Formulations *es puter* (hedonic test) Green tea powder	Fermented black glutinous rice	Appearance	Color	Aroma	Bitterness
4%	25%	7.23±1.48^{ns}	7.51±1.22^{a}	7.03±1.38^{ns}	5.51±2.11^{ns}
	30%	7.09±1.29^{ns}	7.34±1.08^{ab}	6.60±1.59^{ns}	5.46±1.85^{ns}
	35%	6.69±1.88^{ns}	6.86±1.44^{b}	6.51±1.79^{ns}	5.46±2.23^{ns}
The best formula (preference test)		6.89±1.18	7.29±1.09	6.22±1.28	5.47±2.12
		Sourness	Taste	Texture	Overall
4%	25%	5.51±2.20^{ns}	6.29±1.69^{ns}	7.09±1.36^{ns}	6.97±1.52^{ns}
	30%	5.51±2.17^{ns}	6.40±1.88^{ns}	6.63±1.57^{ns}	6.94±1.35^{ns}
	35%	5.37±2.26^{ns}	5.97±1.93^{ns}	6.49±1.58^{ns}	6.49±1.65^{ns}
The best formula (preference test)		5.75±1.74	6.10±1.78	6.65±1.47	6.49±1.42

Data with different letters in column indicated significant differences ($p < 0.05$)
Number of hedonic test panelists = 35
^{ns}superscript in column was not significantly different ($p>0.05$)
Number of preference test panelists = 100

rouxii, Rhizopus oryzae, Mucor indicus, Candida tropicalis and *Saccharomycopsis fibuligera* (Abe *et al*., 2004). It was suggested that the increasing phenolic content might be due to enzymatic hydrolysis by a glycoside hydrolase of insoluble ester type of phenolic acids during fermentation (Ju *et al*., 2009). Table 4 also shows that phenolic content of the three formulations were not significantly different from each other (p>0.05).

Anthocyanin content

Table 4 showed that the anthocyanin content of FBGR (0.36±0.07 mg of total anthocyanin content/100g sample) was higher than unfermented black glutinous rice (0.24±0.01 mg of total anthocyanin content/100g sample) (p<0.05). The increase found in anthocyanin contents of fermented samples in this study were similar to those found by other investigators who worked on other plants. Lee *et al.* (2007, 2008) reported that fermented black beans exhibited higher anthocyanin contents than those of unfermented black beans. Lin and Chou (2006) found that fermentation caused a marked increase in the glycone content (daidzein, glycitein, and genistein), the bioactive isoflavone, and a significant reduction in the content of beta-glucoside isoflavone (daidzin, glycitin, and genistin), compared with the unfermented steamed black bean. Therefore, the action of enzymes such as beta-glucosidase produced by the starter organism during fermentation might be an important factor contributing to the increase of anthocyanin contents of fermented rice. Table 4 also showed that anthocyanin content of three formulations were not significantly different from each other (p>0.05).

Sensory evaluation

Table 5 shows the hedonic result of three selected formulations of *es puter* from 35 panelists. The results showed that none of the attributes showed significant difference except color. According to hedonic result, it showed that formula 4%:25%, 4%:30% and 4%:35% were not significantly different. The results from chemical, phenolic and anthocyanin analysis were not significantly different (p>0.05) as well among three samples, however formula 4%:30% and 4%:35% had higher antioxidant activity than formula 4%:25%. In addition, Formula 4%:30% was more preferable because it had higher hedonic overall score than formula 4%:35%. Therefore, Formula with 4% green tea powder and 30% of FBGR was selected as the best formula.

Consumer test

The majority of panelists were females (71%), the panelists' ages were approximately normally distributed and a median age group of 21–30 years. Most of them were undergraduate students. Table 5 shows the averaged preference test of *es puter* formula by 100 panelists and they liked *es puter* moderately. The data showed that 65% accepted *es puter* whereas the rest did not accept *es puter* due to the bitterness of green tea. It also showed that 53% of panelists said that they would be willing to buy the modified *es puter*.

Proximate analysis

Moisture, protein, fat, carbohydrate, and ash contents of the best formula *es puter* were 66.54±0.25, 1.89±0.06, 3.66±0.75, 0.73±0.02, and 27.18±0.95 g/100 g (%wb), respectively.

Conclusions

The best formula based on physical, chemical quality and sensory analysis of *es puter* was obtained from the blend containing 4% green tea and 30% fermented black glutinous rice. The other ingredients were 51.2% coconut milk, 14% sugar, 0.4% salt, 0.2% CMC and 0.2% GMS, respectively. This formula had overall liking score 6.5 which indicated that the modified *es puter* was liked moderately. In addition, 65% of consumers accepted this product.

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