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EFFECT OF A HEALTHY BISCUIT BY PARTIAL SUBSTITUTION OF GRAPE POMACE AND RICE BRAN POWDER ON SENSORY EVALUATION AND NUTRITION PROPERTIES

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Grape pomace, a byproduct from wine processing, is a combination of grape skin, seed oil and dietary fiber. Grape pomace is considered a rich source of bioactive compounds with various biological properties such as anti-cancer, antioxidant, anti-inflammation and anti-microbial activity. Despite its high nutritional values, Grape pomace is normally discarded or used as animal feed. The purpose of this study is to develop a healthy biscuit by partial substitution of grape pomace and rice bran powder. The moisture, protein, fat, ash, total dietary fiber and carbohydrate contents of grape pomace powder were 3.45, 9.63, 10.78, 5.07, 45.43 and 25.64 g/100 g, respectively. The result indicated the most acceptable control biscuit (100% wheat flour) was modified with Grape Pomace (GP) and rice bran powder (RGP). The most acceptable ratio of grape pomace to wheat flour was 30 to 70. Besides, the most acceptable ratio of grape pomace to rice bran to wheat flour was 30 to 21 to 49. All of the biscuits (control, GP and RGP) were determined for nutrition value, estimated GI and ORAC value. The highest dietary fiber (13.57 g/100 g) and ORAC value (133.71 µmol TE/100 g) was found in the RGP biscuit. On the other hand, the lowest estimated GI was the RGP biscuit. Both RGP and GP biscuits are considered to be the healthy biscuit.

Keywords: Grape pomace, Rice bran powder, Biscuit

INTRODUCTION

Biscuit is a widely bakery product around the world due to the low cost among other processedfoods, varied taste, easy availability and longer shelf life.It is a baked food-product which has wheat flour, sugar, fat, and salt as major ingredients. Biscuit is very high contents of sugar, fats, and calorie but very low amounts of fiber, vitamin, and mineral that make it unhealthy for consumer (Hoseney and Rogers, 1990; and Chevallier *et al.*, 2000). According to WHO recommendation, a healthy diet should contain fruits and vegetables to improve dietary fiber in a diet, less than 10% of total energy intake from free sugar, less than 30% of total energy intake from fat, and less than 5 g of salt (WHO, 2003; and WHO, 2012).

Grape pomace is a waste by-product resulting from the production of wine. It is known to be a natural source of antioxidants which are polyphenolic components such as flavanols, catechins, proanthocyanidins and anthocyanins (Manach *et al.*, 2004). Moreover, it is consists of four major polysaccharides including cellulose, hemicellulose and pectin. The previous study had reported high content of dietary fiber in grape pomace increased catabolic rate and reduced plasma cholesterol by inhibiting the activity of HMG-CoA reductase (Bobek *et al.*, 1998). Several studies

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investigated that grape skins are rich sources of anthocyanins, hydroxycinnamic acids, flavanols, flavonol glycosides and condensed cyanidine-3-glucosides but grape seeds were mainly source of flavanols and proanthocyanidins (Kammerer *et al.*, 2004; Curko *et al.*, 2014). Natural compounds found in grapes have pharmacological and therapeutic affects not only antioxidant but also anti-inflammation and antimicrobial activities (Nassiri-Asl *et al.*, 2009).

Shiraz, also known as Syrah or Hermitage, is one of the most popular grape varieties in the world. It is originally from France. This variety is very important to produce winery products not only France but also Australia and several countries in the world. The flavor is commonly classified as spicy, dark fruit, and berry with different styles produced resulting in the region of origin and winemaking and viticultural decisions (Mayr *et al.*, 2014). Shiraz grape needs really specific weather conditions for suitable growth. It blooms and ripens fairly early and needs really specific moderate heat. Besides, one of the advantages of shiraz is that it has fairly good resistance against most of the diseases in the wines and the soil. Previous studies showed that shiraz grape skins were rich in anthocyanins and flavonols, while the proanthocyanidins are present in the seeds (Downeya and Rochfort, 2008; Cosme *et al.*, 2009). The increasing production yield of grape pomace in northeast region of Thailand and their reported health benefits associated with grape pomace created more experimentation on their chemical compositions for developing further value-added implication in dietary fiber and is suggested to be positively health benefit. There have not been studied in development of healthy biscuit with wine grape pomace. The aim of this study was The purpose of this study is to develop a healthy biscuit by partial substitution of grape pomace and rice bran powder and to evaluate the influence of grape pomace and rice bran powder on sensory evaluation, estimated Glycemic Index (GI), and antioxidant properties of biscuits.

MATERIALS AND METHODS

Preparation of Grape Pomace Powder

Grapr pomace (Shiraz rose) was supplied by Khaoyai Winery, Nakornrachasima, Thailand. The sample was dried in a tray dryer at 60 °C for 24 hours. The dried sample was sterilized at 121 °C for 15 min. Then sample was milled by the dry milling method using the grinder machine (JSP-500, Taiwan) and passed through a sieve at particle size of 200 mesh screen. The gape pomace powder was dried in a tray dryer at 60 °C for 20 min and cooled down to room temperature for 10 min. The grape pomace powder was sealed and stored in polyethylene bag at room temperature (25±5 °C).

Proximated Compositions of Grape Pomace Powder

Proximate compositions (moisture, protein, fat, ash and crude fiber) of SBF were determined by standard methods of analysis (AOAC, 2005). Total dietary fiber was obtained by adding the soluble and insoluble fractions, according to the enzyme matic-gravimetric method of Prosky *et al.* (1998). Carbohydrate content was calculated by difference (100-%moisture-%protein- %fat-%ash-% dietary fiber).

Preparation of Biscuit

The ingredients were prepared according to the recipe present in Table 1. The biscuit was prepared by mixing rice bran oil shortening, sugar and salt in a mixing bowl. The eggs and whipping were added and mixed until slightly firm dough was obtained and rested at 4 °C for 30 min. The dough was manually rolled to form sheet of 0.50 cm thickness. Biscuits were shaped into a round shape with 3.0 cm diameter, baked at 135 °C for 10 min and cooled down to room temperature.

The control biscuit formula was substituted with grape pomace powder at four levels (10, 20, 30 and 40% by weight) in wheat flour. Other ingredients were kept constant (50 g rice bran oil shortening, 50 g sugar and 12 g egg). The biscuits were prepared as described in the control formula.

Table 1: The Ingredients of the Biscuits Recipe

Ingredients (g)	Formulas		
	1	2	3
Wheat flour	110	100	100
Rice bran oil shortening	55	50	50
Sugar	57	50	50
Egg (york)	15	-	-
Egg	-	1	10
Salt	2	0.2	-
Whipping	50	-	-

The grape pomace biscuit was substituted with rice bran powder at four levels (10, 20, 30 and 40% by weight) in wheat flour. Other ingredients were kept constant (50 g rice bran oil shortening, 50 g sugar and 12 g egg). The biscuits were prepared as described in the control formula.

Sensory Evaluation of Biscuit

The sensory evaluations were evaluated by 30 semi-trained panelists. The color, crispness, sweetness and saltiness of biscuits were evaluated using likert scale. Grittiness and overall acceptability using 5-point scoring scale (5 = too gritty, 1 = least gritty and 5 = most acceptable, 1 = least acceptable). The order of presentation of the biscuits was randomized and the samples were coded with the random three-digit numbers.

Determination of Estimated Glycemic Index

The estimated Glycemic Index (estimated GI) of the biscuits was estimated by using the following equation: GI = 39.71 + 0.549 HI (Goni *et al.*, 1997). The Hydrolysis Index (HI) were calculated on the basis of the starch hydrolysis curve (0-120 min) as the percentage of total glucose released over 120 min in comparison to that released from white bread over the same duration.

HI of the biscuits in this study was modified from the method of Granfeldt *et al.* (1992). The biscuit and white bread (reference material) were weighted 100 mg into 50 ml screw-cap tube. The 5 ml of saturated benzoic acid and 10 ml of pepsin solution was added in each tube. All tubes were incubated at 37 °C for 30 min in the water bath shaker (Memmert: SV-142, Germany). After that, 5 ml of sodium acetate buffer was added in each tube and incubated at 37 °C for 2 min. Then, 5 ml of enzyme mixtures was added in each tube and incubated at 37 °C. After 20, 40, 60, 80, 100 and 120 min, the hydrolyzed sample was transferred for 200 µl into the test tube containing 4 ml of 98% ethanol. The 100 µl of solution at 20, 40, 60, 80, 100 and 120 min was taken into test tube containing 2 ml of glucose-oxidase peroxidase kit (GOD-PAP), then incubate in a water bath at 45 °C for 20 min. After that, 250 µl of sample was added and then measured at 510 nm by UV/VIS spectrophotometer.

Determination of Antioxidant Activity

The ORAC assay was slightly modified according to the method of Ou *et al.* (2001). The extracted samples were mixed with fluorescent solution (30 nM) in a 96–well black plate before being incubated for 15 minutes at 37 °C. After the incubation, AAPH (19.125 nM), a peroxy l radical generator, was added to the reaction mixture and immediately started the reaction. The fluorescence intensity was monitored for 90 min using a micro plate reader (Synergy HT multi-detection micro plate reader, Bio–Tek Instruments, Inc., Winooski, VT), with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Loss of fluorescence indicates an extended damage of the reaction as the peroxyl radical being induced by AAPH. Trolox, a water-soluble analogue of vitamin E (3.125, 6.25, 12.5, 25, 50 or 100 µM), was used as a control standard, and phosphate buffer (75 mM, pH 7.4) was used as a control blank. The results were calculated based on the differences in areas under the sodium fluorescent decay curve (AUC), and were expressed as µmol Trolox Equivalence (TE) per 1 g sample. The AUC can be calculated as:

$$AUC = 0.5 + f_1/f_0 + f_2/f_0 + f_3/f_0 + \ldots + (0.5)f_{90}/f_0$$

where f_0 is the initial fluorescence reading at 0 min and $f_{1, 2, \ldots, 90}$ is the fluorescence reading at 1, 2, …, 90 min.

Statistical Analysis

A Randomized Complete Block Design (RCBD) was conducted for sensory evaluation. A Completely Randomized Design (CRD) with three replications was conducted for estimated glycemic index and antioxidant activity. Statistical program, SPSS software for Windows version 18.0 (SPSS Inc., Illinois, USA) was used to perform the statistical analysis. Duncan's multiple range tests was used to differentiate between the mean values. P < 0.05 was considered statistically significant differences.

RESULTS AND DISCUSSION

Proximated Compositions of Grape Pomace Powder

Total dietary fiber was the major component in grape pomace powder, followed by carbohydrate protein fat and ash, respectively (Table 2). Similar observation was reported by Sousa *et al.* (2014) who found that protein and total dietary fiber were 8.49 and 46.17 g/100 g, respectively. Grape pomace powder is source of fiber. Therefore, the biscuit that formulated by substituting wheat flour with grape pomace powder could increase fiber content and decreased GI of the product. Fiber can inhibit starch degradation and hence alter the mount of sugars released during the digestion (Tudorica *et al.*, 2002).

Table 2: Proximate Compositions and Microbiological of Grape Pomace Powder

Compositions	g/100g	g/100g*
Moisture	3.45±0.02	3.33±0.04
Protein	9.63±0.04	8.49±0.02
Fat	10.78±0.03	8.16±0.01
Ash	5.07±0.02	4.65±0.05
Total dietary fiber	45.43±0.03	46.17±0.08
Carbohydrate	25.64	29.2
Microbiological		
Total plate count	ND	-
Yeasts and molds	ND	-

Note: ND = Not Detected

Source: Sousa et al. (2014)

The microbiological showed that the grape pomace powder has characteristics acceptable for human consumption.

Sensory Evaluation

The results shown in Table 3, it was noted that the scores of crispness, saltiness and overall acceptability among three formulas was significantly different ($p \leq 0.05$), except the scores of color and sweetness ($p > 0.05$). The biscuit formula 3 was selected as the control formula as it had highest score for overall acceptability ($p \leq 0.05$).

Table 3: Sensory Attribute Scores of the Biscuit (Control Formula)

Attributes	Formulas		
	1	2	3
Color*	2.56±0.57	2.60±0.72	2.46±0.72
Crispness*	2.47±0.68^{b}	3.33±0.54^{a}	3.30±0.66^{a}
Sweetness*	3.10±0.88	3.26±0.69	2.96±0.56
Saltiness*	2.10±0.71^{b}	2.27±0.74^{b}	2.83±0.65^{a}
Overall acceptability**	3.10±0.66^{c}	3.50±0.66^{b}	4.03±0.72^{a}

Note: ^{a,b,c} Means followed by different letters within the same row are significantly different ($p \leq 0.05$). * Five-point just-about-right scale (5 = too much, 3 = just-about-right, 1 = too few). ** Five-point scoring scale (5 = most acceptable, 1 = least acceptable).

The sensory scores of the biscuits with different levels of grape pomace powder are shown in Table 4. The scores of color and grittiness of all biscuits were significantly different ($p \leq 0.05$). The scores of color and grittiness increased as the levels of grape pomace powder increased. The results indicated that increased levels of grape pomace powder resulted in the low acceptability score. The scores of crispness and overall acceptability of the biscuit with 40% of grape pomace powder was significantly differently ($p > 0.05$) from the biscuit with 30% of grape pomace powder. Gluten is the main structure-forming protein in flour, and is responsible for the elastic characteristics of dough, and contributes to the appearance and crumb structure of baked products. Gluten removal results in major problems for bakers, and currently, many gluten-free products available on the market are of low quality, exhibiting poor mouthfeel (Arendt *et al.*, 2002; and Gallagher *et al.*, 2004). In this experiment, the biscuit substituted with 30% of grape pomace powder was selected for the next study.

The score for sensory evaluation of the biscuits given in Table 5 reveal that there are significant differences ($p \leq 0.05$) betweentreatments for sensory attributes color, crispness, grittiness and overall acceptability. The highest score of color was the biscuit with 40% rice bran powder ($p \leq 0.05$). With increasing the level of substitution the color of the biscuit turn form light purple to dark purple, leading to lower acceptance. The biscuit with 40% rice bran powder had the lowest score of crispness ($p \leq 0.05$). Overall acceptability was determined on the basis of quality scores obtained from the evaluation of color, crispness, sweetness, saltiness and grittiness of the biscuits. The biscuit with 40% rice bran powder had the lowest score of overall acceptability ($p \leq 0.05$). The score of overall acceptability of the biscuit with 30% rice bran powder was not significantly different ($p > 0.05$) from the biscuit with 0, 10 and 20% rice bran powder. In order to get the best nutritive value, the biscuit with 30% rice bran powder was selected to next study.

The decrease in overall acceptability was due to decrease in crispness score. Gluten removal results in the crispness that is important element in the quality of the biscuit, directly affecting consumer acceptance (Gaines, 1993). Then, the biscuit with 30% rice bran powder was selected to determine the nutritive value, estimated GI and ORAC value.

Estimated Glycemic Index (GI)

The Area Under the Curve (AUC) values were determined

Table 4: Sensory Attribute Scores of the Biscuit with Five Grape Pomace Powder Levels (0, 10, 20, 30 and 40%)

Attributes	Grape Pomace Powder: Wheat Flour				
	0.069444444	0.479166667	0.888888889	30:70	40:60
Color*	2.4 ± 0.77^{c}	2.97 ± 0.89^{b}	3.63 ± 0.81^{b}	4.33 ± 0.76^{a}	4.77 ± 0.73^{a}
Crispness*	3.03 ± 0.61^{a}	3.00 ± 0.64^{a}	2.73 ± 0.64^{ab}	2.63 ± 0.56^{b}	2.37 ± 0.56^{c}
Sweetness*	3.07 ± 0.45^{ab}	3.23 ± 0.50^{a}	3.27 ± 0.52^{a}	2.93 ± 0.83^{b}	2.93 ± 0.45^{b}
Saltiness*	2.60 ± 0.77	2.50 ± 0.86	2.53 ± 0.94	2.63 ± 0.85	2.50 ± 0.68
Grittiness**	2.30 ± 0.75^{c}	2.77 ± 0.50^{b}	2.87 ± 0.63^{b}	3.33 ± 0.71^{a}	3.57 ± 0.57^{a}
Overall acceptability***	3.70 ± 0.84^{a}	3.77 ± 0.82^{a}	3.67 ± 0.71^{a}	3.53 ± 0.63^{a}	3.10 ± 0.88^{b}

Note: [a,b,c,d] Means followed by different letters within the same column are significantly different ($p \leq 0.05$), * Five-point just-about-right scale (5 = too much, 3 = just-about-right, 1 = too few). ** Five-point scoring scale (5 = too gritty, 1 = least gritty). *** Five-point scoring scale (5 = most acceptable, 1 = least acceptable).

Table 5: Sensory Attribute Scores of the Biscuit with Five Rice Bran Powder Levels (0, 10, 20, 30 and 40%)

Attributes	Rice Bran Powder: Wheat Flour				
	0.069444444	0.479166667	0.888888889	30:70	40:60
Color*	2.50 ± 0.90^{c}	3.27 ± 0.52^{b}	3.57 ± 0.56^{b}	4.30 ± 0.83^{a}	4.27 ± 0.83^{a}
Crispness*	2.83 ± 0.53^{ab}	2.63 ± 0.61^{b}	3.10 ± 0.70^{a}	3.20 ± 0.75^{a}	1.33 ± 0.75^{c}
Sweetness*	2.83 ± 0.53	2.97 ± 0.76	2.83 ± 0.75	2.83 ± 0.67	2.80 ± 0.74
Saltiness*	2.53 ± 0.73	2.67 ± 0.80	2.80 ± 0.48	2.47 ± 0.76	2.47 ± 0.68
Grittiness**	2.93 ± 0.82^{b}	3.03 ± 0.76^{b}	3.10 ± 0.75^{b}	3.23 ± 0.74^{b}	4.02 ± 0.75^{a}
Overall acceptability***	3.47 ± 0.77^{a}	3.73 ± 0.78^{a}	3.70 ± 0.82^{a}	3.57 ± 0.83^{a}	2.20 ± 0.76^{b}

Note: [a,b,c] Means followed by different letters within the same column are significantly different ($p \leq 0.05$). * Five-point just-about-right scale (5 = too much, 3 = just-about-right, 1 = too few). ** Five-point scoring scale (5 = too gritty, 1 = least gritty). *** Five-point scoring scale (5 = most acceptable, 1 = least acceptable).

and compared to the AUC of white bread to obtain the HI of each biscuits. The AUC, HI and estimated GI value as shown in Table 6. The white bread (as standard) value was adjusted mathematically to represent HI value of 100, and the calculation was applied to biscuits in the same experiment.

The estimated GI of biscuits of control, GP, RGP and white bread were 86, 78, 62 and 95, respectively. The results demonstrated that GP and RGP had lower GI than the control formula. The GP and RGP biscuits had lower GI than control, its containing dietary fiber. The dietary fiber could inhibit the swelling and gelatinization of the starch, and hence reduce starch digestibility (Brennan *et al.*, 2004). The fiber

Table 6: Area Under Curve, Hydrolysis Index and Estimated Glycemic Index of Biscuit

Formulas	AUC	HI	Estimated GI
Control[1]	11339	85	86
GP[2]	9265	70	78
RGP[3]	5486	41	62
White bread	13307	100	95

Note: [1] Control = 100% wheat flour. [2] GP = 30% grape pomace powder and 70% wheat flour. [3] RGP = 30% grape pomace powder, 21% rice bran powder and 49% wheat flour.

mix was added to flour used in the preparation of biscuits markedly reduce its GI (Franca *et al.*, 2008). In this study the substitution of wheat flour with grape pomace and rice bran powder in the control formula. The biscuit could substitute 30% grape pomace powder and 21% rice bran powder for wheat flour in the control formula. The GI value of the biscuit was 62 which classified as medium GI (Foster-Powell *et al.*, 2002).

The ORAC Value of the Biscuit

The ORAC value of the biscuits is shown in Table 7. The RGP biscuit showed the highest value of ORAC ($p \leq 0.05$). The ORAC value of the GP biscuit was higher than the control biscuit ($p \leq 0.05$). The result indicated that the substitution of grape pomace powder was significantly increased the ORAC value of the biscuit.Moreover, Goufo *et al.*, reported that rice bran contained antioxidant properties resulting from ORAC assay (105.5 ± 80.15 mmol trolox/100 g) (Goufo *et al.*, 2014) because rice bran is the richest source of phenolic acids in rice (Tian *et al.*, 2004; Finocchiaro *et al.*, 2007). Ashoush *et al.* indicated that mango peels and seed kernels powder replacing wheat flour increased phenolic acid contents from 3.84 to 24.37 mg/g biscuit. It is possible to enhance the nutritional quality and improve antioxidant property of the biscuit (Ashoush and Gadallah, 2011).

The biscuit was formulated with grape pomace and rice bran powder could enhance the ORAC value and total dietary fiber intake of consumers towards the recommended level. The biscuit could label as “Good source” of fiber because thetotal dietary fiber of this biscuit was ranging between 13-18% of RDI (FDA, 1998). Moreover, the dietary fiber of grape pomace and rice bran powder could reduce the estimated GI of the biscuits.

Table 7: The ORAC Value of the Biscuits

Formulas	Content (µmol TE/100 g)
Control[1]	11.56 ± 0.27^{c}
GP[2]	81.56 ± 7.86^{b}
RGP[3]	133.71 ± 4.02^{a}

Note: [a,b,c] Means followed by different letters within the same column are significantly different ($p \leq 0.05$). [1] Control = 100% wheat flour. [2] GP = 30% grape pomace powder and 70% wheat flour. [3] RGP = 30% grape pomace powder, 21% rice bran powder and 49% wheat flour.

CONCLUSION

Based on this study, the developed biscuit with grape pomace and rice bran powder are considered healthier biscuit because it is a good source of total dietary fiber, high antioxidant capacity. Moreover, the RGP is classified as medium eGI. The suggestion to consume the biscuit is to divide the biscuits into moderate portion size (25-30 g) and should be eaten between meals as a healthy snack.

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