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Research paper

Development of Snack Bar from Black Soybean and Black Rice for Breastfeeding Mothers

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Abstract

Breastfeeding mothers need more nutrients compared to mothers who are not breastfed to maintain quality and quantity of human milk they produce. Black soybean and black rice contain carbohydrate, protein, fat, and other nutrients that needed by breastfeeding mothers. The purpose of this study was to develop a snack bar for breastfeeding mothers. Snack bars were made from three ratio variations of black soybean flour: black rice flour (30:70, 50:50, and 70:30) with baking temperature 150 ^{0}C for 20 minutes. They were then subjected to sensory analysis (taste, flavor, color, texture, aftertaste, and overall); physical analysis (hardness, color); and chemical analysis (nutritional analysis, genistein, and daidzein). The results showed that snack bar with ratio 30:70 (black soybean: black rice) was preferred by panelists. Its level of hardness was 6.10±0.01 N. Its color was showed in color index L* = 25.04±1.71, a* = 6.05±0.70, b* = 3.89 ± 0.71, chroma = 6.66±0.25, and hue = 54.70±2.40. One serving (30 gram/serving) of this snack bar contained 16.28 grams of carbohydrate; 3.10 grams of protein; and 6.05 grams of fat. It could suffice 4.98 % of total energy based on Recommended Dietary Allowance of Indonesian breastfeeding mother per day. Meanwhile, the content of daidzein and genistein were 0.09 mg/serving and 1.35 mg/serving, respectively. In conclusion, snack bar from black soybean and black rice can be used as a complementary food for breastfeeding mothers due to its nutrient content and acceptability.

Keywords: *Breastfeeding mothers, snack bar, black soybean, black rice.*

1. Introduction

Breastfeeding plays an important role in the infant's growth. Breast milk is the best food for infant due to balanced nutrition that could not be obtained from other food such as formula milk. Usually, breast milk is given exclusively for six months after birth. Breastfeeding women need 500 calories more than nonbreastfeeding women to produce a sufficient amount of milk, so that, breastfeeding women need more nutrition from their daily food and additional food [1].

These days, milk is the most popular additional food for breastfeeding women. However, snack devoted for breastfeeding mother, especially snack bar, are still rarely found in the market. The snack bar is a solid food made from the various mixtures such as nuts, dried fruits, and cereals which are combined with binding materials such as sugar, honey, and chocolate. The snack bar has promising potential in the market these past ten years because of its convenience and nutritional value [2].

Black soybean and black rice could be processed into a snack bar to fulfill the nutritional needs of a breastfeeding mother. Black soybean is widely consumed due to its nutritional value. It contains phytoestrogen isoflavone, a bioactive compound that has a benefit in human health. Isoflavone comes in aglycone form and glycoside form, which is more abundant in soybean. However, glycoside form could not be absorbed by the human body. Therefore, it must be changed to aglycone form first by microflora in the gut [3]. Daidzein and genistein are two types of aglycone form of isoflavone that can be found in black soybean. Isoflavone possesses the ability as a galactagogue, substance which can increase milk production and improve milk quality. Isoflavone has a similar structure with estrogen so they can bind to isoform α and β from estrogen receptor. This binding induces prolactin hormone that responsible for the milk production [4].

Black rice has some nutritional advantages over common rice, such as a higher content of protein, vitamins and minerals, lipid, and anthocyanin [5]. Anthocyanin is a pigment that plays a role in red, purple, and black color in plants including black soybean and black rice. Anthocyanin comes in various type and has higher antioxidant activity than vitamin C and vitamin E. Anthocyanin stability strongly affected by temperature, light intensity, and pH [6]. The most abundant anthocyanin in black soybean and black rice is cyanidin-3-glycoside [7,8]. This study was carried out to get the optimum formula, sensory acceptance, physical, and chemical properties of the snack bar from black soybean and black rice for breastfeeding mothers.

2. Materials and methods

2.1. Materials

Black soybean was purchased from Gunung Kidul, Yogyakarta, Indonesia, and black rice was purchased from Rahong village, Cianjur, West Java, Indonesia. Vanilla flavor, butter, almond, walnut, raisins, choco chip, and sugar were purchased from the local market in Tangerang, Indonesia. Methanol, acetonitrile, acetic acid, DMSO, genistein, and daidzein standard (HPLC grade) were derived from Sigma-Aldrich (France).

2.2. Formulation of the snack bar

Black soybean and black rice were ground using food processor then sifted with 80 mesh sieve. Three variations of black soybean: black rice ratio were used in this research. The first variation consisted of 15 g black soybean flour: 35 g black rice flour (30:70), the second variation was 25 g black rice flour: 25 g black soybean flour (50:50), the third variation was 35 g black rice flour: 15 g black soybean flour (70:30). Each dough was made by mixing 25 g sugar, 4 g vanilla flavor, 15 g butter, and 50 g mix flour. Afterward, 5 g almond, 5 g walnut, 5 g choco chip, 5 g granola, and 10 g raisins were added to the dough and mixed well. 30 g of weight was shaped into 8 cm x 2 cm x 1.5 cm. The bars were baked in an oven for 20 minutes at 150^{o}C.

2.3. Sensory evaluation

Sensory evaluation using 9-hedonic scale was conducted to know the consumer acceptability. 30 untrained breastfeeding women between 23-36 years old were used as the panelists. Color, taste, texture, flavor, aftertaste, and overall were the sensory attributes which be tested. A commercial snack bar was used as a control.

2.4. Physical analysis

Color analysis was performed by a colorimeter (Minolta CR-400), and hardness level was performed by texture analyzer (TA-XT 2i).

2.5. Proximate analysis

Water content was measured by oven method, protein content was measured by micro-Kjeldahl method, lipid content was measured by Soxhlet extraction method, ash content was measured by dry ashing method, crude fiber content was measured by acid-base method, and carbohydrate content was measured by difference method [9].

2.6. Isoflavone analysis

Table 1: Sensory evaluation result

Formula	Color	Flavor	Texture	Taste	Aftertaste
1	6.46^{a}±1.43	6.79^{a}±1.42	6.86^{a}±1.24	6.82^{a}±1.44	6.19^{a}±1.63
2	5.79^{a}±1.62	6.43^{a}±1.76	5.61^{b}±1.57	5.84^{b}±1.93	5.38^{a}±1.95
3	5.43^{a}±1.60	6.32^{a}±1.63	5.93^{ab}±1.49	5.86^{ab}±1.38	5.54^{a}±1.95
Control	6.43^{a}±1.67	6.07^{a}±1.74	6.25^{ab}±1.65	6.25^{ab}±1.92	6.14^{a}±1.84

Formula (black soybean: black rice) 1= 30:70; 2= 50:50; 3= 70:30. Values are presented as means and standard deviations. Values followed by different superscript letters in a column are significantly different from each other ($p < 0.05$).
Scale 1 = extremely dislike, 2 = very dislike, 3 = dislike, 4 = rather dislike, 5 = neutral, 6 = rather like, 7 = like, 8 = very like, 9 = extremely like
Control: commercial chocolate flavor snack bar

Two g of each sample was extracted by adding 10 ml acetonitrile and 2 ml HCl 0.1 N. The mixture then mixed for 2 hours at room temperature. After that, the samples were centrifugated 4500 rpm for 10 minutes. The supernatants were filtered with Whatman paper No. 42. Then, filtered supernatants were evaporated in the vacuum oven for 16 hours. The evaporated samples were dissolved in 10 ml methanol. Then, the methanol solution was prepared to HPLC injection by filtered 1.5 ml sample with microfilter 0.45 µm. 50 µl sample was injected to HPLC (Agilent 1100) and eluted under gradient conditions. The mobile phase consisted of 2 solutions. Solution A was 0.1% acetic acid glacial in distilled water, and solution B was 0.1% acetic acid glacial in acetonitrile. The solvent gradient was programmed as follows: 85% A/15% B in 0 minutes and 65% A/35%B in 0-20 minutes. The solvent flow rate was 0.5 ml/min, and the chromatogram was recorded at 254 nm. Genistein and daidzein standards were diluted in DMSO in the concentration of 5, 10, 25, 50, 100, and 200 mg/l [8].

2.7. Statistical analysis

Data were reported as mean ± standard deviation. The means of all parameters were examined for significance using ANOVA (IBM SPSS 2.0) with Tukey's significant difference post-hoc test. Statistical significance was carried out at $p < 0.05$.

3. Results and discussion

3.1. Sensory evaluation

The variations of sensory attributes among samples are presented in Table 1. Panelists perception of color, flavor, and aftertaste attributes in all samples have no statistically significant differences ($p > 0.05$). High temperature used in the baking process produced caramelization and Maillard reaction. Caramelization formed carbonyl compound such as ketone and aldehyde meanwhile Maillard reaction produced a volatile compound which could produce more flavor. Caramelization and Maillard reaction also formed brownish color in snack bar [11]. Panelists perception of texture attribute in formula 1 was significantly higher than formula 2 ($p < 0.05$). Higher black rice content in formula 1 produced softer texture which was more acceptable than formula 2. Black rice made the snack bar has a softer texture. The previous study stated that softer texture of bread and food product was preferred by panelists [12]. Panelists perception of taste in formula 1 was significantly higher than formula 2 ($p < 0.05$). Formula 2 contained higher black soybean. Black soybean content in the snack bars caused a bitter taste. The bitter taste is not liked by panelists. The bitter taste was caused by the activation of bitter taste receptor hTAS2Rs [13]. In some snack bars, the taste of the snack bar came with an astringent aftertaste. This astringency occurred due to phenolic acid and isoflavone from black soybean [14].

3.2. Hardness level

The hardness level of snack bar is presented in Figure 1. Formula 3, which contained higher of black soybean, was the hardest snack bar among all samples with statistically significant differences ($p < 0.05$). This hard texture was caused by high protein content in the snack bar. The previous study said that soy protein could increase the hardness level of bread significantly [15]. Maillard reaction occurred during the baking process also increased hardness level. Maillard reaction caused protein cross-linking that formed a more rigid and compact structure [16].

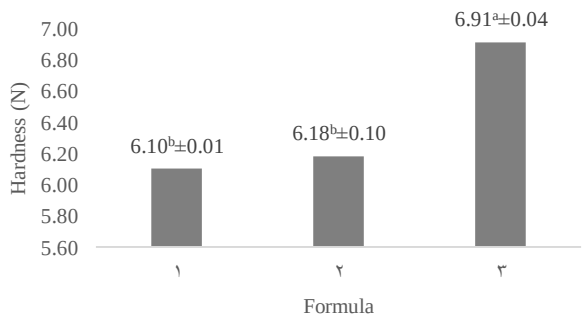

Fig. 1: Hardness analysis of snack barFormula (black soybean: black rice) 1: 30:70; 2: 50:50; 3: 70:30. Values are presented as means and standard deviations. Values followed by different superscript letters in a different bar are significantly different from each other ($p < 0.05$).

3.3. Color analysis

The color analysis result is presented in Table 2. The number of black rice used in the snack bar caused the lower L values obtained. Lower L values indicated a darker color in the sample. Formula 1 was significantly darker than formula 3. In a* value, formula 1 had the highest value with a significant difference. The higher content of black rice used in snack bar produced darker snack bar due to the quantity of anthocyanin. Black rice has more anthocyanin that is evenly spread in aleurone, pericarp, and seed coat [5]. Anthocyanin in black soybean could be found only in the seed [17]. Anthocyanin, which is responsible for the red, purple, blue, and black color in plants, is unstable and could change because of pH, light intensity, and temperature. Baking process and storage condition also affected anthocyanin stability resulted in the reddish color in snack bar [6].

Table 2: Color analysis result

Formula	L*	a*	b*	C	H
1	25.04^{b}±1.71	6.05^{a}±0.70	3.89^{a}±0.71	6.66^{a}±0.25	54.70^{a}±2.40
2	27.40^{ab}±0.61	2.55^{b}±0.28	4.24^{a}±0.21	4.95^{a}±0.16	58.95^{a}±1.53
3	30.19^{a}±0.42	2.81^{b}±0.08	4.65^{a}±0.30	5.43^{a}±0.30	58.90^{a}±0.95

Formula (black soybean: black rice) 1= 30:70; 2= 50:50; 3= 70:30. Values are presented as means and standard deviations. Values followed by different superscript letters in a column are significantly different from each other ($p < 0.05$). L* : black-white : 0-100; a* : green-red : -80 - +80; b* : blue-yellow : -70 - +70; c : chroma; H^0 : hue

Table 3: Proximate composition of snack bar

Formula	Moisture (% wb)	Ash (% db)	Protein (% db)	Lipid (% db)	Carbohydrate (% db)	Fiber (% db)
1	12.07^{b}±0.75	1.79^{c}±0.02	10.33^{c}±0.09	20.15^{a}±2.11	54.25^{a}±2.30	2.23^{a}±0.15
2	12.28^{a}±0.56	2.03^{b}±0.03	12.58^{b}±0.09	19.33^{a}±1.09	50.90^{a}±0.92	2.88^{a}±0.47
3	12.68^{a}±0.36	2.35^{a}±0.08	15.35^{a}±0.81	21.95^{a}±0.60	45.33^{b}±2.12	2.48^{a}±0.28
USDA	<10	1.89	8	20.39	63.82	-

Formula (black soybean : black rice) 1: 30:70; 2: 50:50; 3: 70:30. Values are presented as means.

Table 4: Nutritional composition per serving (per 30-gram snack bar)

Formula	Water (ml)	Ash (gram)	Protein (gram)	Fat (gram)	Carbohydrate (gram)	Fiber (gram)
1	3.38	0.54	3.10	6.05	16.28	0.67
2	3.68	0.61	3.77	5.80	15.27	0.86
3	3.80	0.71	4.61	6.56	13.60	0.74
RDA	2950	-	76	88	364	-

Formula (black soybean : black rice) 1: 30:70; 2: 50:50; 3: 70:30. Values are presented as means.
Amount per snack bar (g) = (proximate (%) x weight per snack bar (30 g))/100.

Table 5: Energy conversion per serving snack bar (per 30-gram snack bar)

Formula	Protein		Lipid		Carbohydrate		Total energy	
	(cal)	%RDA	(cal)	% RDA	(cal)	% RDA	(cal)	% RDA
1	12.39	4.08	54.41	6.87	65.10	4.47	131.90	4.98
2	15.09	4.96	52.18	6.59	61.08	4.20	128.35	4.84
3	18.42	6.05	59.00	7.45	54.39	3.74	131.81	4.97

Formula (black soybean : black rice) 1= 30:70; 2= 50:50; 3= 70:30. Values are presented as means.

3.4. Proximate analysis

The proximate composition of three snack bars is presented in Table 3. The moisture content in formula 1 significantly different ($p < 0.05$) among the others because of the water content in black rice as the main raw material was higher than in black soybean. Ash and protein content values were significantly different among samples ($p < 0.05$). Higher black soybean concentration also made higher protein and ash content in the snack bar. Soybean has a high concentration of protein and ash as well so that soybean usually use as a protein source in many food products [18]. Protein and ash content of the snack bars were higher than USDA's standard. Therefore, the formulated snack bars could be an alternative source of protein and mineral. Lipid and fiber content was no significant difference among samples.

Based on proximate composition, nutrition composition or nutrition fact per serving could be counted and presents in Table 4. According to the Recommended Daily Allowance for Indonesian breastfeeding mother in the first six months of breastfeeding, a 30 g snack bar could fulfill around 4.9% of the total energy needed per day (Table 5).

3.5. Isoflavone analysis

Isoflavone daidzein and genistein content per serving is presented in Table 6. The quantity of daidzein and genistein varied between 0.09-12.05 mg per serving for daidzein and 1.35-2.57 mg per serving for genistein. Raw black soybean flour was used as a control. Genistein content was higher than daidzein content in all samples including control. The previous study also stated that genistein was higher than daidzein both in raw soybean and soybean product [19]. The difference number of isoflavone between the control and the samples was mainly caused by the heating process and also storage condition that can reduce isoflavone quantity [20].

It was also known that daidzein is more unstable and more easy to degrade at a higher temperature than genistein [21]. Daidzein and genistein could act as a galactagogue in several ways. Daidzein could promote breastmilk production by upregulating prolactin receptor and STAT5 (signal transducer and activator of transcription 5) signaling which has an important role in milk production [22]. Genistein was known to have the ability to stimulate mammary hyperplasia [23].

Tabel 6: Daidzein and genistein concentration in the snack bar

Formula	Daidzein (µg/g)	Daidzein per serving (mg)	Genistein (µg/g)	Genistein per serving (mg)
1	2.86	0.09	44.93	1.35
2	8.07	0.24	53.31	1.60
3	12.05	0.36	85.66	2.57
Control	73.33	-	358.54	-

*Formula (black soybean: black rice) 1= 30:70; 2= 50:50; 3= 70:30. Values are presented in means

4. Conclusion

Snack bar could be made from black soybean and black rice. Snack bar with a ratio of 30:70 (black soybean: black rice) was the most optimum snack bar due to the sensory acceptance. Physical properties of snack bar showed that 30:70 snack bar was the softest snack bar, while its color was the darkest and the reddest snack bar. Its level of hardness was 6.10±0.01 N. Its color was showed in color index L* = 25.04±1.71, a* = 6.05±0.70, b* = 3.89 ± 0.71, chroma = 6.66±0.25, and hue = 54.70±2.40. One serving (30 gram/serving) of this snack bar contained 16.28 grams of carbohydrate; 3.10 grams of protein; and 6.05 grams of fat. It could suffice 4.98 % of total energy based on Recommended Dietary Allowance of Indonesian breastfeeding mother per day.

Meanwhile, the content of daidzein and genistein were 0.09 mg/serving and 1.35 mg/serving, respectively. All formulation of snack bar could be an alternative food for breastfeeding women with good nutritional value. The result could help snack production to make specialized snack bar for breastfeeding women. Further research is needed to ensure the clinical effectiveness of the snack bar.

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