ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Characteristics of Biscuits with Antioxidant Made from Elicitated Black Soybean Sprout.

Saptya Fajar Pertiwi*, Sudarminto Setyo Yuwono, and Erni Sofia Murtini.

Department of Food Science and Technology, University of Brawijaya, Malang, East Java, Indonesia.

ABSTRACT

This research aimed to obtain biscuit with antioxidant activities that meet the requirement as functional food. The main ingredients of biscuit were elicited black soybean sprout flour and red rice flour, and the formulation was performed using linear programming. In order to meet criterion, minimum amount of elicited black soybean sprout flour added to formulation was 48.02%, yielding antioxidant biscuit containing isoflavon 28.39 mg/100g. Incorporation of 80% elicited black soybean sprout flour resulted in sensorially unacceptable product, assuming the consequence of strong beany flavor and bitter after taste. This study concluded that proportion of elicited black soybean sprout flour 48.5 g and red rice flour 52.5 g showed sensorially acceptable biscuit with antioxidant activity that met the requirement.
Keywords: biscuit, isoflavon, antioxidant, soybean, and functionality.

**Corresponding author*

INTRODUCTION

Black soybean (*Glycine soja* L.) is one of local food sources purportedly functional foods due to high antioxidative compounds such as flavonoid, anthocyanin and isoflavones, particularly daidzein and genistein [7]. The genistein and daidzein were reported to exert antioxidant properties [14].

High demand in functional food leads to tremendous attraction for development of high antioxidant products as functional food [1]. Elicited black soy bean sprout flour can be developed as ingredient for biscuit. However, since it is not carbohydrate source, there is a need to use other sources such as red rice flour to obtain desirable characteristics of biscuit. High amylose content (≥25%) plays important role in the formation of dry biscuit texture [6]. Like wise, pigmented rice such as red and black rice have been popular due to high content of antioxidant and phenolic compounds [25], but black rice production is still limited as compared to red rice. Thus, red rice flour was chosen to incorporate in the biscuit formulation for enhancement of antioxidant properties. The use of elicited black soy bean sprout flour and red rice flour is expected to produce high antioxidant biscuit as functional food, as well as improve their added value. Linear programming is selected to determine the biscuit formulation due to its low cost.

MATERIAL AND METHODS

Materials:

Black soybean, ethanol 70%, distilled water, DPPH 0.2 M (2.2 – diphenyl – 1 – pikryl – hidrazyl), gallic acid, follin-ciolcateu 50%, Na_2CO_3 5%, quercetin, $NaNO_2$ 5%, $AlCl_3$ 10%, NaOH 1M, standard daidzein and genistein, asetonitril 20%, and acetic acid.

Tools:

Rotary shaker, vacuum rotary evaporator, cabinet dryer, spectrophotometer, HPLC: column (LiChrospher RP C-18 4 mm x 250 mm x 5 µm), wavelength 260 nm, temperature 30 ^{0}C, diode array detector (DAD).

Preparation of Elicitated Black Soybean Sprout Flour:

Soybean was cleaned and soaked in chitosan solution (650 ppm) for 8 h. The elicitated soybean was sprouted for 60 h at 30 ^{0}C. The sprout soybean was dried at 50 ^{0}C for 8 h for preparation of elicitated black soybean flour.

Preparation of Biscuit Dough and Baking:

Formulation was determined using linear programming based on isoflavone content of 0.0167%, 0.0501% and 0.17%. All dry ingredients were evenly mixed and incorporated with other ingredients as outlined in Table 1.

The dough was then sheeted to a thickness of 0.3 cm and cut to a diameter of 3 cm. The biscuits were baked at 115 ˚C for 30 minutes in baking oven. They were cooled and stored at aluminium foil-layered plastic bag, which protected them against light. The data were average values of 5 replications.

Extraction of Sampels [22]:

Biscuit was grinded to obtain biscuit powder. The sample powder (1 g) was added with ethanol 70% (different ethanol volumes were used each analysis: antioxidant 100 ml; total phenol 100 ml; flavonoid 30 ml; and isoflavone 5 ml). The extraction was conducted in closed erlenmeyer with aluminium foil. The mixture was shaked at 300 rpm for 3 hours in room temperature. The extraction was then carried out in 12 hour of dark. The extract was filtered using whatman paper, and the supernatant was transferred into another tube. For isoflavone analysis, the extract was evaporated using rotary evaporator at 40 °C. The concentrated extract was stored at 4 °C in dark room for further analysis.

Antioxidant Activity of Biscuit [23]:

The sample extract (2 ml) was placed in reaction tube, added with 1 ml of DPPH, and evenly mixed. The mixture was then kept at dark room for 30 minutes. The absorbance was detected at wavelength 517 nm. Antioxidant activity was calculated as ascorbic acid equivalent (AAE).

Total Phenol of Biscuit [16; 18; 24]:

A tube containing sample extract (2 ml) was added with 5 ml of distilled water and 0.5 ml of Follin-Ciolcateu 50%, and then incubated for 5 minutes. Na_2CO_3 5% (1 ml) was added, and the mixture was homogenized and incubated for 1 hour. The absorbance was detected at wavelength 725 nm using spectrophotometer.

Determination of Biscuit Flavonoid [3]:

A tube containing sample extract (1 ml) was added with 4 ml of distilled water and 0.3 ml of $NaNO_2$ 5%. The mixture was homogenized and incubated for 5 minutes. The mixture was then added with 0.3 ml of $AlCl_3$ 10%, 2 ml of NaOH 1 M, and 2.4 ml of distilled water. The mixture was subsequently mixed and incubated at 30 minutes. The absorbance was detected at 410 nm using spectrophotometer.

Determination of Isoflavone [15]:

Concentrated extract (1000 mg) was added with 10 ml of solvent, and mixed using ultrasonic for 15 min. The solution was then filtered using nylon 0.2 µm into vial, and stored for analysis.

Sensory Evaluation [2]:

Sensory analysis using Rate All That Apply (RATA) for evaluation atribut intensity of biscuit with Antioxidant. Quantitative descriptive sensory analysis of biscuits was performed by a panel of 100 panelis untrained. The quality descriptors and weighting coefficients to a three-score range evaluation scale were chosen by panelis according to the procedure included (1 = maximum; 3 = minimum intensity).

Experimental Design:

Complete randomized design was used, which consist of independent variables (biscuit formula) determined by linier programming. Independent variables were antioxidant activity, total phenol, and flavonoid. The experiment was performed at 5 replications. The average data were expressed in table and compared. Sensory data were evaluated with analysis of General Linier Model (GLM). The best treatment was determined using multiple attribute Zeleny.

RESEARCH RESULTS

Chemical Composition of Flours:

Characteristics of elicitated black soybean flour and red rice flour were presented in Table 2.

Table 1: Composition of Antioxidant Biscuit

Compositions (g)	Formulation		
	F1	F2	F3
Black soybean sprout flour	14.3	48.52	80
Red rice flour	85.7	51.48	20
Sugar	7.9	7.9	7.9
Salt	0.2	0.2	0.2
Vanilla paste	0.2	0.2	0.2
Baking powder	0.2	0.2	0.2
Egg yolk	3	3	3

Margarine	13.3	13.3	13.3
Total	**124.8**	**124.8**	**124.8**

Linier Programming and modified from Zucco *et al.*, 2011

Table 2: Chemical Composition of Elicitated Black Soybean Flour and Red Rice Flour

Parameters	Elicitated Black Soybean Flour	Red Rice Flour
Moisture (%)	8.102 ± 0.308	9.109 ± 0.134
Antioxidant Activity (mg AAE/g)	1.734 ± 0.00189	1.468 ± 0.00064
Total Phenol (mg GAE/g)	3.422 ± 0.081	0.188 ± 0.0203
Flavonoid (mg QE/g)	1.674 ± 0.016	1.772 ± 0.055
Isoflavon (mg/g)	3.504	-
- Genistein	2.2489 ± 0.002	-
- Daidzein	1.255 ± 0.005	-
Protein (%)	50.645 ± 0.134	10
Fat (%)	15.92 ± 0.664	1
Carbohydrate (%)	20.508 ± 0.453	73
Ash Content (%)	4.825 ± 0.078	6.891

Profile of Antioxidant Activity, Total Phenol, and Flavonoid

The end product of this study was biscuit with antioxidant properties. The antioxidant activity was calculated according to standard curve of ascorbic acid equivalent, and expressed as mg AAE/g. Total phenol was determined based on standard curve of galact acid equivalent and expressed as mg GAE/g, while flavonoid was determined using standard curve of quercetin, and expressed as QE/g. The result of these experiments was outlined in Table 3.

Table 3 showed that treatments (F1, F2, and F3) indicated significant effects (p-*value* ≤ 0,05) on antioxidant activity (0.72 ± 0.030^{b}, 0.76 ± 0.037^{ab} and 0.83 ± 0.052^{a} mg AAE/g, respectively), total phenol (2.01 ± 0.005^{c}, 2.12 ± 0.003^{b}, and 2.25 ± 0.013^{a} mg GAE/g, respectively), and flavonoid (1.62 ± 0.018^{c}, 1.59 ± 0.019^{ab}, and 1.55 ± 0.039^{b} mg QE/g, respectively).

Table 3: Profile of Antioxidant Activity, Total Phenol, and Flavonoid of Biscuits

Parameters	Formulation		
	F1	F2	F3
Antioxidant activity (mg AAE/g) db	0.72 ± 0.030^{b}	0.76 ± 0.037^{ab}	0.83 ± 0.052^{a}
Total Phenol (mg GAE/g) db	2.01 ± 0.005^{c}	2.12 ± 0.003^{b}	2.25 ± 0.013^{a}
Flavonoid (mg QE/g) db	1.62 ± 0.018^{c}	1.59 ± 0.019^{ab}	1.55 ± 0.039^{b}

The different superscript following the values means significant difference (ANOVA with Tukey test at α = 0.05).

Isoflavone Content in Different Formulation of Biscuit

Presence of isoflavone daidzein and genistein in samples was detected using HPLC instrument, as presented in Table 4.

Table 4 exhibited that content of daidzein and genistein was increased with increasing of elicited black soybean sprout flour more than 48.02%. The isoflavone content of each biscuit (F1, F2, and F3) was 13.13, 28.39 dan 31.63 mg/100 g, respectively.

Table 4: Isoflavone Content of Biscuits (per 100 g)

Biscuit	Isoflavone (%)		Total Isoflavone (mg/100 g) db
	Daidzein	Genistein	
F1	0.011 ± 0.002	0.041 ± 0.002	13.13
F2	0.019 ± 0.001	0.067 ± 0.001	28.39
F3	0.014 ±0.000	0.076 ± 0.002	31.63

Biscuit F1 (14.3 g of elicited black soybean sprout flour: 85.7 g of red rice flour), Biscuit F2 (48.5 g of elicited black soybean sprout flour: 52.5 g of red rice flour), Biscuit F3 (80 g of elicited black soybean sprout flour: 20 g of red rice flour).

Sensory Profile of Biscuit

The intensity of sensory attributes was assessed using rate all that apply (RATA), which cover 3 samples of biscuit made from different proportions of elicited black soybean sprout flour and red rice flour (14.3g:857 g; 48.52g:51.48 g; and 80g:20g) (Figure 1). The result was presented in Table 5.

Table 5: Average Values Sensory Attributes Biscuit at Different Flour Proportions

Sensory Attributes	**Flour Proportion (elicited black soybean sprout flour : red rice flour)**		
	14.3 : 85.7	**48.5 : 52.5**	**80 : 20**
Sweetness	1.44^{a}	1.17^{a}	0.27^{b}
Bitter	0.06^{b}	1.77^{a}	1.93^{a}
Umami	1.71^{a}	1.16^{b}	0.30^{c}
Beany flavor	0.25^{c}	1.90^{a}	1.55^{b}
Rice flavor	0.09^{c}	1.69^{a}	0.69^{b}
Sweet aroma	1.84^{a}	1.64^{a}	0.19^{b}
Compactness	0.19^{b}	0.30^{b}	0.68^{a}
Crunchy	1.61^{a}	1.58^{a}	0.45^{b}
Darkness	0.41^{b}	1.79^{a}	1.65^{a}
Neutral	0.50^{a}	0.54^{a}	0.15^{b}
Lightness	1.26^{a}	0.95^{b}	0.15^{c}
Bitter after taste	0.34^{b}	1.85^{a}	1.68^{a}
Sweet after taste	0.41^{a}	0.41^{a}	0.12^{b}

The different superscript following the values means significant difference (ANOVA with Tukey test at α = 0.05).

DISCUSSION

Characteristics of Biscuit

The results revealed that flour proportion of elicited black soybean sprout flour and red rice flour influenced content of antioxidant, total phenol, and isoflavone. The presence of isoflavone in black soybean was responsible for antioxidant activity [13]. Genistein and daidzein were isoflavones that showed antioxidant properties [8], and they were contributed to functional properties in both soybean and derived products [13]. Therefore, biscuit containing elicited black soybean sprout flour resulted in higher antioxidant activity.

Phenolic compounds were flavonoids, divided into such groups as flavones, flavon-3-ol, flavonone, flavan-3-ol and anthocyanin, which also contribute as antioxidant agents [11]. Types of phenolic compounds in bean can be observed from its peel. Black soybean peel is account for black color, and it is also included in elicited black soybean sprout flour. Presence of peel is strongly associated with high phenolic compounds. Various pigments such as purple, red, and black were responsible for indicators of phenolic compounds [11].

Bioactive compounds such as anthocyanin and proanthocyanidin were contributors for flavonoids in red rice flour. Anthocyanin was a pigment that accounts for natural antioxidant properties in red rice [19]. Proanthocyanidin in red rice was 3.168 mg CE/mg extract [12]. High concentration of proanthocyanidin in red rice may cause high flavonoid content in biscuit made from 85.7 g of red rice flour.

In addition, isoflavone content of elicited black soybean sprout flour was higher than that of biscuits. This discrepancy may result from such factors as flour proportion, heating process, and oxidation. As antioxidant agent, isoflavone was easily able react with free radicals for oxidation, since it had high reactivity [17]. Oxidation occurred due to formation of oxygen singlet, induced by UV radiation and ionization radiation [17]. Oxygen singlet was oxygen with higher reactivity compared to ground state oxygen, and it was able to accelerate oxidation reaction in food, even though the food was processed at low temperature [10][5].

Sensory Evaluation

Figure 1 exhibited 13 sensory attributes detected by panelists. The attributes were evaluated by these scales: 1 = strong; 2 = medium; 3 = weak. The sensorially acceptable biscuit characteristics included desirable taste, crunchy, fragrant flavor, and yellowish-brown color [4]. Figure 1 presented that proportion of elicited black soybean sprout flour and red rice flour (14.3 g:857 g; 48.52g:51.48 g; and 80 g:20 g) had 15 sensory attributes. Flour proportion at 80 g:20 g showed the highest score of such attributes as bitter taste, compactness, dark color, and bitter after taste. The score of sensory attributes was decrease hard and wiry texture in three biscuit formulations. The combination may show no effects on changes in these texture properties.

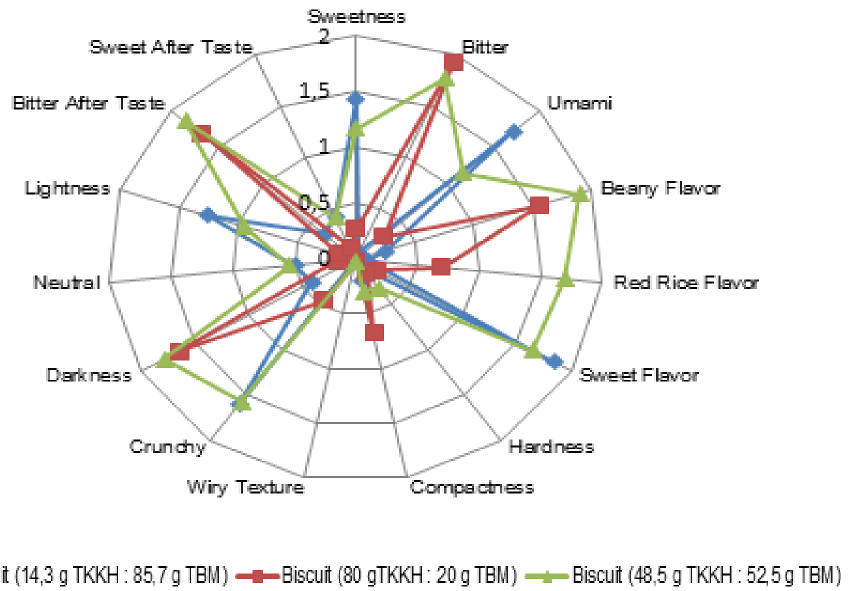

Figure 1:. Spider Chart of Sensory Attributes for Biscuits

High protein flour required water to avoid hydration for production of hard texture, thus presence of protein was responsible for reduction of dough hardness [26]. Hidrocoloid addition was not required in preparation of gluten free biscuit due to its high water binding capacity [21]. Rice flour contained high starch fraction, thus improving water absorption [20]. In addition, combination of two types of flour in our experiment may retard starch retrogradation, yielding anti-staling effects. Staling is undesirable effect on baking product since it leads to hard texture. Starch could be modified through substitution of OH groups in amylose and amylopectin with particular compounds to produce esterified starch. The compounds widely used were acetic groups, hydroxypropyl, and octenyl succinate that were applicable for reduction of starch retrogradation [9].

CONCLUSION

Higher proportion of elicited black soybean sprout flour in the formulation resulted in biscuit with higher antioxidant activity, total phenol, flavonoid, and isoflavone. In order to meet standard as functional food (BPOM 2011), minimum amount of isoflavone required in biscuit (each serving) was 5 mg, thus addition of elicited black soybean sprout flour was no less than 48.02%, yielding antioxidant biscuit with isoflavone of 28.39 mg/100 g. Proportion of elicited black soybean sprout flour and red rice flour (48.52 g : 51.48 g) resulted in the best biscuit in term of bioactive compounds and sensory attributes. Our data revealed that the best biscuit had antioxidant activity 0.761 mg AAE/g, total phenol 2.124 mg GAE/g, flavonoid 1.593 mg QE/g, and isoflavone 28.39 mg/100 g. Based on sensory evaluation, the best biscuit formulation resulted in strong sweet taste, low bitter taste, strong umami taste, low beany flavor, low rice and sweet flavor, slight crunchy, high lightness, low darkness, and low bitter after taste as well as strong sweet after taste.

REFERENCES

[1] Aparicio-Sangulian A, Sayago-Avendi G S, Vargas-Torres A, Tovar J, Ascensio-Otero E T and Bello-Perez A L. Journal of Food Composition and Analysis 2007; 20: 175-181.

[2] Ares G, F Bruzzone, L Vidal, R S Cadena, A Gimėnez, B Pineau, D C Hunter, A G Paisley and S R Jaeger. Journal Food Quality and Preference 2014; 36: 87-95.

[3] Chang C, Yang M, Wen H and Chem. Journal Food Drug Analaysis 2002; 10: 178-182

[4] Ertanto, T. *Reaksi maillard pada produk pangan*. IPB. Bogor 2008.

[5] Herawati and Syafsir Akhlus. Kinerja (Bht) Sebagai Antioksidan Minyak Sawit Pada Perlindungan Terhadap Oksidasi Oksigen Singlet. Jurusan Kimia, Institut Teknologi Sepuluh Nopember. 2006

[6] Hormdok R and Noomhorm A. Journal of Food Science and Technology. 2007; 40 (10): 1723-1731.

[7] Huang X, C Weixi and X Baojun. Journal Food Chemistry 2014; 143: 268-276.

[8] Jha H C, S Kiriakidis, M Hoppe and H Egge. Antioxidative Constituents of Tempe. In. Sudarmadji dkk. (eds.) 1997.

[9] Kusnandar Feri. *Jurnal FATETA IPB* 2010.

[10] Min D B and Boff J M. Journal Food Science and Food Safety 2002; 1: 58-64.

[11] Mir A S, Bosco Don J S, Shah A M and Mir M M. Journal Food Chemistry 2016; 191: 139-146.

[12] N Thitipramote, P Pradmeeteekul, J Nimkamnerd, P Chaiwut, P Pintathong and N Thitilerdecha. Journal International Food Research 2016; 23 (1): 410-414.

[13] Nurrahman. *Jurnal Aplikasi Teknologi Pangan* 2015; 4(3): 89-93.

[14] Paucar-Menacho M Luz, Berhow A M, Mandarino J M G, Chang Y K and Mejia E Gonzalez de. Journal Food Research International 2010; 43: 1856–1865.

[15] Penalvo J L, T Nurmi and H Adlercreutz. Journal Fppd Chem 2004; 87: 297-305.

[16] Santoso J, N Aryudhani and S H Suseno. Jurnal Kelautan Nasional 2009; 2: 109-118.

[17] Sayuti K and Yenrina R. Antioksidan alami dan sintetik. Andalas University Press 2015; ISBN: 978-602-8821-97-1.

[18] Sharma G N, S K Dubey, N Sati and J Sanadaya. International Journal of Pharmaceutical and Clinical Research 2011; 3(2): 27-29.

[19] Suardi. *Jurnal Litbang Pertanian* 2005; 24(3).

[20] Sudha L M, Srivastava K A, Vetrimani R and Leelavathi K. Journal of Food Engineering *2007;* 80: 922-930.

[21] Torbica Aleksandra, H Miroslav and H Tamana D. Journal Food Research International 2012; 48: 227-283.

[22] Xu B J and Chang S K C. Journal of Food Science 2007; 72(2): 159-166.

[23] Yan L Y, Teng L T and Jhi T J. Journal Sunway Academic 2006; 3: 9-20.

[24] Yangthong M, N Hutadilok-Towatana and W Phromkunthong. Journal Plant Foods Human Nutrition 2009; 64: 218-223.

[25] Zaupa M, Ganino T, Dramis L, and Pellegrini N. Journal Food Structure 2016; 7: 6-12

[26] Zucco F, Borsuk Y and Arntfield D S. Journal Food Science and Technology *2011*; 44: 2070-2076.