Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development
Vol. 17, Issue 3, 2017
PRINT ISSN 2284-7995, E-ISSN 2285-3952

BIOACTIVE COMPOUNDS AND FUNCTIONAL PROPERTIES OF SOME FLOUR COMPOSITES IN THE MANUFACTURING OF FUNCTIONAL FOOD PRODUCTS

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Abstract

Composite flour from banana corm, sweet potato, and black soybean has rich nutrition content and contains bioactive compounds, which potentially beneffficial for human health if consumed in proper amount. It is important to know composite flour properties prior apply to food product. The objective of this research was to determine the bioactive compounds and functional properties of composite flour that can be applied for functional food products. The method used was an experimental method by descriptive analysis with two replications. Composite flour made by mixture of 55 part banana corm flour, 45 part of sweet potato flour and black soybean flour added as much 20 percent of the weight of banana corm and sweet potato flours. The result showed that the composite flour had bioactive compounds which contained 9.09 g/ 100g of total phenols, 1.62 g/ 100g of tannins, 171.35 mg/ 100g of isoflavones, and IC_{50} 979.64 ppm of antioxidant activity (very weak). The amylograph characteristic pasting involves initial temperature of gelatinization 76.62^{0}C, 1304.5 cP of peak viscosity, 930.0 cP of hot pasta viscosity, 1267.5 cP of cold pasta viscosity, 374.5 cP of breakdown viscosity and 337.50 cP of setback viscosity. Therefore, the composite flour from banana corm, sweet potato, and black soybean has rich nutritions to be used as functional food.

Key words: *amylograph, bioactive compounds, composite flour, functional food.*

INTRODUCTION

The use of wheat as raw material for food industry in Indonesia remains high, so it becomes an important problem that needs to be handled immediately. One effort that can be achieved is by reducing the use of wheat flour through some mixture of flours or often called composite flour. Composite flour is flour made from two or more foodstuffs in order to obtain characteristics which have appropriate materials for processed products, or to obtain specific functional properties.. Local commodities that are potentially upraised in the production of composite flour are banana corm, sweet potato and black soybean.

The production of banana in 2015 amounted to 7.29 million tons from 6.86 million tons in 2014, it means this production increased by 9.35%. Based on these data banana corm produced tends to increase and quite a lot, however it's not optimally utilized at recent time. On the other side, sweet potato production in 2015 is 2.29 million tons while in 2014 is 2.38 million tons; it means that was decreased by 3.57 %; and also the soybean production in 2015 is 963.18 thousand tons while in 2014 is 954.99 thousand tons of dry beans [3].

In general, banana is grown only for the fruit, and the leaf use for wrapping only. Banana plants bear the fruit only once in a lifetime, after that the trunk and corm are cut down and left-over. So far, banana corm has been only considered as waste by many people. The banana corm just thrown away and some people use it as animal feed, but it is only for some farmer. Appropriately, banana corm can be used optimally in the form of flour. The excellence of using it as flour is the carbohydrates content in the form of oligosaccharides such as fructo oligosaccharides (FOS) and gluco oligosaccharides (GOS), dietary fiber and bioactive components such as tannins [11].

Besides banana corm, sweet potato is one of the local commodities which is potential to be used as the manufacture of composite flour. Sweet potato var. Ase Kuning not only contains high carbohydrate (85.68%), but also β-carotene functions as an antioxidant and provitamin A that can be converted into vitamin A in the body.

As well as the two above commodities, black soybean can also be used in the production of composite flour to fulfill protein content (39.09%). Black soybean is one of the local commodities which is very potential to be used as functional product because it contains essential amino acids, vitamin E, saponins and rich in antioxidants such as flavonoids, isoflavones and anthocyanin [15].

That three kind flours have a good advantage of chemical properties, bioactive components and functional properties, combined into composite flour, it will have good nutritional value. The purpose of this study was to determine bioactive components and functional properties contained in the composite flour that can be utilized in the manufacture of functional food products.

MATERIALS AND METHODS

Materials

Materials used in this study were banana corm, sweet potato var. Ase Kuning and black soybean clone AKIBE-1 from Faculty of Agriculture Unpad, distilled water and sodium bisulfite, NaCl, and DPPH also chemical substances for analysis.

Research Method

Research method used was descriptive research method conducted with two replications, for each sample of the study including:

A : Banana Corm Flour
B : Sweet Potato Flour
C : Soybean Flour
D : Composite Flour

Making Composite Flour

The composite flour composition, namely mixing 45.8% of banana corm flour, 37.5% of sweet potato flour and 16.7% black soybean flour [13].

Phenolic Test with Spectrophotometer Method [1]

A total of 3 grams of each flour was put into a 50 ml measuring flask and precised with distilled water (60.000 ppm). Then, took 0.5 ml solution put into a 25 ml flask, and added 0.5 ml Folin Ciocalteu shaken for 5 minutes and added 10 ml Na_2CO_3, precised with distilled water, homogenized and let stand for 1 hour. Absorbance was measured at a wavelength of 750 nm. Gallic acid was used as standard in various concentrations.

Tannins Test with Spectrophotometer Method [1]

A total of 3 grams of each flour was added to 50 ml flask and matched with distilled water (60,000 ppm). Then, took 0.5 ml solution and put into a 25 ml measuring flask, then added Folin-denis 1.25 ml, shaken for 5 minutes and added 2.5 ml Na_2CO_3 and accorded with distilled water, homogenized and let stand for 1 hour, then absorbance was measured at a wavelength of 760 standard nm. Tanat acid was used as standard in various concentrations.

Isoflavones Analysis with HPLC Method [1]

A total of 5 grams of each flour was added into Erlenmeyar 250 ml, added 40 ml extraction solution, methanol: water (80: 20). Then, it was entered into a water bath shaker at a temperature of 65ºC for 2 hours. It's cooled and added 3 ml of 2 N NaOH, shaken for 10 minutes and added 1 ml of glacial acetic acid, shaken back and then removed quantitatively into a 50 ml measuring flask, then it was filtered with Whatman filter paper No. 42, pipetted 5.0 ml filtrate to 10.0 mL volumetric flask and added 4.0 ml aquabidest, matched with methanol water (1: 1) and mixed it carefully. Pipetted 1.0 ml into a centrifuge tube then centrifuge for 5 minutes at 7000 rpm. The supernatant was pipette into the vial and injected into the chromatography column

Standards used were 10 mg daidzin, 10 mg genistin, 10 mg daidzein, 10 mg genestein, 10 mg glycitein, put into 50 ml flask. Then entered the 10 mg vial glycitin to 50 mL volumetric flask, diluted with methanol until limit mark. Main standard solution was pipetted for 0.25 ml into 50 ml measuring flask and added water

methanol 1: 1 until limit mark, then it's injected into the chromatography column.
HPLC condition: HPLC Shimadzu, isocratic, sanpel volume 20 ul, column: C 18, eluent: methanol and glacial acetic acid (98: 2), detector: SPD 10A, flow rate 0,4 ml / min, temperature 25-27°C, wavelength 260 Nm and LC10AD pump.

Calculation:

$$\text{Isoflavones(ug/g)} = \frac{(\text{Sample area} - \text{standard area} \times \text{standard concentration} \times \text{fp})}{\text{sample weight}}$$

Determination of Antioxidant Activity with DPPH Method [6]

Two grams of each flour was put into a reaction tube, and then added 0.5 ml DPPH 160 ppm (dissolved in methanol). Then the sample was homogenized and incubated for 30 minutes in a dark room and read the absorbance at 516 nm wavelength with UV-VIS spectrophotometer. Standard used was ascorbic acid in varying concentrations. The absorbance value obtained was used to determine inhibition value of sample through the following calculation formula:

$$\%\ \text{inhibition} = \frac{\text{A blank} - \text{A sample}}{\text{A blank}} \times 100\%$$

Notes :
A blank = blank count absorbance
A sample = sample count absorbance
% Inhibition = percentage of free radical inhibition capacity.

The results of the calculations were included in regression equation to obtain IC_{50} values, that was the value obtained from the calculation when the percent inhibition reached 50%.

Analysis of Amilograph Characteristics [14]

Material of 3.5 grams was prepared and then inserted into a canister. Distilled water was added as much as 25 grams. The canister was put inside the tool and began taking measurements by pressing tower button. The sample was heated to a temperature of 50^0C and maintained for 1 minute. The sample was heated from 50^0C up to 95^0C then it was maintained for 6 minutes in the 95^0C temperature. The sample was cooled to a temperature of 50^0C and maintained for 3 minutes (using Standard 2).

RESULTS AND DISCUSSIONS

Bioactive Compounds

Analysis of bioactive compounds is taken to provide an overview of any bioactive compounds contained in the flours tested. The test results of bioactive compounds in banana corm flour, sweet potato flour, black soybean flour and composite flour is shown in Table 1. The test results showed that banana corm flour, sweet potato flour, soybean flour and composite flour contained phenolic compounds.
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Three general types of phenolic compounds are flavonoids, phenolic acid and polyphenols and generally analyzed as total phenols. From the above table, banana corm flour gained the highest phenols content (9.09 g/ 100g) compared to sweet potato and black soybean flours.

Table 1. The test results of bioactive compounds in banana corm flour, sweet potato flour, black soybean flour and composite flour

Sample	Phenol Content (g/100g)	Tannin Content (g/100g)	Total Iso-flavone (mg/100g)
Banana Corm Flour	9.09	11.59	-
Sweet Potato Flour	4.00	1.32	-
Black Soybean Flour	3.03	2.23	230.56
Composite Flour	8.14	1.62	171.35

Source: Own calculation

Composite flour contained phenols (8.14 g/ 100g) lower than banana corm flour because the composite flour was a mixture of 45.8 % of banana corm flour, 37.5 % of sweet potato flour and 16.7% of black soybean flour. Banana corm contains active compounds such as flavonoids (8.18%), saponins (6.70%) and tannins (4.38%) [9]. Phenolic compounds on sweet potato act as antioxidants. Black soybean is a food rich in antioxidants, this is caused by the presence of non oxidative components, primarily from phenols group such as isoflavones and anthocyanin pigments.

In line with the phenolic compounds, banana corm flour had the highest tannins content (11,59 g/ 100g) than sweet potato flour and black soybean flour. Composite flour contained tannins compound of 1.62 g/ 100g. Tannins are an active compound of secondary metabolite which are known to have some benefits as astringent, anti-diarrhea, anti-bacterial and antioxidant. Tannins are component of organic substance that are very complex, consisting of phenolic compounds which are difficult to separate and difficult to crystallize, precipitate protein from it solution and bound with the protein.

The test results showed that banana corm flour and sweet potato flour did not contain isoflavones, whereas black soybean flour contained isoflavones amounted to 230.56 mg/ 100g and composite flour amounted to 171.35 mg/ 100g. Isoflavone compounds on composite flour derived from black soybean flour. Isoflavones belong to the group of flavonoids and polyphenolic compounds found in many fruits, vegetables, and grains. In black soybean var. Mallika, isoflavones consist of genistin (0.65 mg/g) and daidsein (3.67 mg/g) [8]. Soybean flour and soybean grain contain isoflavone compounds ranged between 128.35 -298.95 mg/ 100 grams [7].

The resulted composite flour contained bioactive compounds such as phenols, tannins and isoflavones, it means the three flours are complementing each others besides increasing activity value of the combination. Bioactive compounds in composite flour are antioxidant compounds, therefore the composite flour can be applied on manufacturing of functional food products.

Antioxidant Activity

The test results showed that banana corm flour, sweet potato flour, soybean flour and composite flour had a value of antioxidant activity means having the ability to inhibit DPPH radical. Antioxidants are compounds that can counteract or reduce the negative impact of oxidant. Antioxidants work by donating one electron to oxidant compounds so that the oxidant compound activity can be inhibited [17].

Table 2. Antioxidant activity of banana corm flour, sweet potato flour, black soybean flour and composite flour

Samples	IC_{50} (ppm)
Banana corm flour	422.16
Sweet Potato Flour	5418.96
Black Soybean Flour	20781.14
Composite Flour	979.64

Source: Own calculation

Antioxidants are also able to inhibit oxidation reactions by binding free radicals and highly reactive molecules subsequently the damage cells can be prevented. The highest antioxidant activity was found in banana corm flour with IC_{50} value of 422.16 ppm (weak). Antioxidant activity of composite flour had IC_{50} value of 979.64 ppm (very weak). Antioxidant activity in the composite flour is allegedly due to the presence of active compounds of phenols, tannins and isoflavones. All flour either in single or mixed form showed IC_{50} valuesabove 200 ppm. Antioxidant activity is classified very strong if the IC_{50} value is less than 50 ppm, strong if IC_{50} is between 50 –200, weak if IC_{50} is between 200-600 and very weak if the IC_{50} is greater than 600. The smaller the value IC_{50} the stronger theantioxidant activity.

IC_{50} valuesfor composite flour showed very weak antioxidant capabilities [6]

Amilograph Characteristic

Gelatinization initial temperature showed that the highest initial temperature of gelatinization retained by black soybean flour for 95.00^{0}C, sweet potato flour of 80,47^{o}C, flour composite of 76,62^{o}C, and banana corm flour 50,27^{o}C. The temperature obtained is the temperature when the first time of viscosity from each flour begins to rise.

The high initial temperature of gelatinization is due to the amylose content which is higher than the amylopectin. Amylose is able to hold hydrogen bonds with another amylose or with amylopectin forming configurations that is difficult to destroy because there are many hydrogen bonds within the granules so that it requires more energy [4]. Banana corm flour has amylose of 36.40% and amylopectin of 63.60% [13], and sweet potato flour has amylose of 16.86-21.58% and amylopectin of 20-28% [2]. Tapioca flour has high amylopectin value amounting to 83%, coincidentally banana corm flour has similar characteristics to tapioca flour.

Table 3. Amilograph characteristics of banana corm flour, sweet potato flour, black soybean flour and composite flour

Amilograph Characteristics	Banana Corm Flour	Sweet Potato Flour	Black Soybean Flour	Composite Flour
Initial Temperature of Gelatinization (ºC)	50.27	80.47	95.00	76.62
Peak Viscosity (cP)	4615.00	945.00	9.50	1304.50
Hot Pasta Viscosity (cP)	2039.00	266.50	1.50	930.00
Cold Pasta Viscosity (cP)	7767.50	350.00	-2.50	1267.50
Breakdown (cP)	2576.00	679.00	8.00	374.50
Setback (cP)	5728.50	83.50	-4.00	337.50

Source: Own calculation.

Banana corm has higher amylose (36.40%) [13] than sweet potato 16.86-21.58%) [2] but the gelatinization initial temperature was on the contrary, this happened supposedly in the process of banana corm flour making, some of the starch are already experiencing gelatinization so on amilograph test for gelatinization initial temperature of banana corm flour was lower than sweet potato, soybean and composite flour. High amylose content tends to have high gelatinization temperature and water absorption level, it is due to amylose bond stronger than amylopectin bond.

The high initial temperature of gelatinization is not only affected by amylose and amylopectin but is also affected by protein and fat in the food. Soybean has the highest protein amounted to 35-38% and fat by 20% [16], and banana corm flour has protein amounted to 0.60% and sweet potato has protein content of 2.85% [13]. During heating, protein will be denatured around gelatinization temperature. Protein leads to hindrance of water migration process into starch granules thereby increases the gelatinization temperature.

High fat content may interfere process of starch gelatinization, this is because fat is able to create a complex with amylose so the amylose can not depart from the starch granules. As a result, a greater energy is required to release amylose so the initial gelatinization temperature will be higher. Meanwhile, low gelatinization temperature will be beneficial because it can save cooking energy.

Peak viscosity parameter is the ease of dough in the cooking and shows the strength of dough formed by gelatinization during processing in application of the foodstuffs. The measurement result of peak viscosity showed that the highest values were obtained by banana corm flour 4,615.00 cP, composite flour 1,304.50 cP and sweet potato flour 945.50 cP. The low viscosity peak of black soybean flour because it contains starch and carbohydrates. Consequently at the test time it did not produce gel clotting (no gelatinization), only produced a liquid looked like soymilk. A decrease in viscosity peak indicates a decline in ability of the dough to expand.

The high viscosity peak is affected by amylopectin level contained in a material, this because the branch of amylopectin structure increases the ability to bind water and form viscosity. Amylopectin is starch component which is responsible for granules expanding process [3].

Composite flour had lower viscosity peak value than banana corm flour but higher than sweet potato flour, this was due to the portion of banana corm flour in composite flour was more than sweet potato flour, so the peak viscosity of composite flour was between banana corm and sweet potato flours. Composite flour had a peak viscosity value of 1304.50 cP, it shows that the composite flour dough strength rated 1304.50 cP compared with other flours. Composite flour has much better expanding capacity than others except with banana corm flour.

Based on the amilograph characteristics on peak viscosity, it's known that composite flour had a peak viscosity of 1304.00cP, to determine whether the composite flour is suitable for food product then it is necessary to do conversion to BU unit that generated 621.19

BU. Based on amilograph properties, raw material with peak viscosity <500 BU is suitable for wet products, peak viscosity of 500-1,000 BU is suitable for wet and semi product, and peak viscosity > 1,000 BU is suitable for extrusion products (products that expand) such chiki and crackers [5]. From the above calculation, it is known that the composite flour is suitable for semi-wet products such as cookies, crackers and others.

Hot pasta viscosity is an index of the cooking easiness and reflects weakness of granules to expand. Decrease of hot pasta viscosity value is generally followed by an increase in breakdown. The breakdown viscosity is obtained from reduction of peak viscosity with hot pasta viscosity. Breakdown viscosity or decrease during the heating showed stability of pasta during the process [10]. However, at certain temperature, the decrease of hot pasta viscosity is not always accompanied by increase of breakdown. If the hot pasta viscosity and pasta peak viscosity decrease proportionally, the breakdown would likely remain.

Hot pasta viscosity showed that banana corm flour had the highest value of 2,039.00 cP, composite flour of 930.0 cP, sweet potato flour of 266.50 cP and soybean flour of 1.50 cP. The measurement result of breakdown viscosity showed that banana corm flour amounted to 2,576.00 cP, sweet potato flour 374.0 cP and composite flour 679.0 cP. Breakdown viscosity on soybean flour was the least of 8.0 cP.

Increase in breakdown viscosity value shows that the starch can not stand to heating and stirring [4]. In this research, sweet potato flour was stable on heating process, soybean flour only contributed to add protein value. Therefore, composite flour gained fair breakdown viscosity value compared with banana corm flour and sweet potato flour, this was due to the nearly balance parts of banana corm flour and sweet potato flour mixture in the composite flour. Amylopectin level affects on breakdown viscosity, this is because the structure of amylopectin branching leads to less stable against heating.

Retrogradation tendency can be seen from the cold pasta viscosity and setback. Setback or change in viscosity during cooling is obtained from the difference between the cold pasta viscosity with hot pasta viscosity. The higher the value of setback shows the higher the tendency to form a gel (increase viscosity) during the cooling. The high value of setback indicates a tendency for the occurrence of retrogradation. Retrogradation is a re-crystallization process of starch that has experienced gelatinization, while syneresis is a liquid discharge of a gel of starch [18]. During cooling, re-merging starch molecules mainly amylose will result a formation of gel structures and viscosity including cold pasta viscosity.

Cold pasta viscosity showed that cold pasta viscosity of banana corm flour was the highest, followed by sweet potato flour then composite flour (Table 3). The increase in viscosity during the cooling determine the tendency of starch re-merging which reflects tendency of product to retrogradated [12].

Banana corm flour had a setback viscosity of 5,728.50 cP, followed by composite flour of 337.50 cP, sweet potato flour of 83.50 cP. Setback viscosity value in soybean flour was -4 cP, this is due to the absence of peak viscosity, consequently the setback viscosity value can not be calculated.

Banana corm flour gained higher setback viscosity value compared to others. It shows that the retrogradation process is getting stronger. Soybean flour had the lowest setback viscosity, it indicates that soybean flour is difficult to experience the retrogradation process after cooling. Setback viscosity is the parameter of recrystallization of gelatinized starch during the cooling.

CONCLUSIONS

Composite flour, banana corm flour, sweet potato flour, and black soybean flour produced had bioactive compounds such as phenols, tannins, and isoflavones. The composite flour had bioactive compounds which contained 9.09 g/ 100g of total phenols, 1.62 g/ 100g of tannins, 171.35 mg/ 100g of isoflavones, and IC_{50} 979.64 ppm of antioxidant activity (very weak). The amylograph characteristic of composite flour involved initial temperature of gelatinization 76.62^{0}C, 1304.5 cP of peak viscosity, 930.0 cP of hot pasta viscosity, 1267.5 cP of cold pasta viscosity, 374.50 cP of breakdown viscocity and 337.50 cP of setback viscosity. From bioactive compounds and functional properties, the composite flour is suitable to be used in the manufacture of functional food such as biscuits, cookies and others.

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