International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.08, pp 257-266, 2018

Phytochemical Composition And Antioxidant Activity Of Composite Flour From Banana, Corn And Sago

Edi Suryanto[1]*, Lidya Irma Momuat[1] And Frenly Wehantouw[2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Science, Sam Ratulangi University, Manado, Indonesia
[2]Integrated Laboratory, Sam Ratulangi University, Manado, Indonesia

Abstract : Banana, corn and sago are well recognised as excellent source of phytochemicals and macronutrients. It has been developed composite flour that has high antioxidant and synergist effect on the activity of three plants.The objective of this study was to evaluate phytochemical content and to determine antioxidant activity in composite flour from banana, corn and sago.The extracts from composite flour were measured for phytochemical content and antioxidant activities using spectroscopic method.The results showed that extraction of cosolvent mixtures had significant influence on phytochemicals content and antioxidant activity. The highest total phenolic content was found in 80% methanol and 80% ethanol for F1 and F2, whereas F3 was found in 80% acetone in composite flour. In addition, acetone 80% in F3 showed the highest total carotenoid content followed with F2 and F1. Conversely, 80% acetone in F2 showed the highest free radical scavenging activity comparable that of F1 and F3.The results also showed that 80% acetone in F3 were found highest total antioxidant capacity than F1 and F3. The results suggest that 80% acetone to extract phenolic phytochemicals in composite flour from banana, corn and sago.
Keywords : Composite flour, banana, corn, sago,phytochemicals, antioxidant.

Introduction

The banana plant is classified as a great perennial treelike, tropical herb of the genus *Musa* and the family Musaceae. Banana is one of fruit that much comsumptionin the worldwide for better fresh (common banana)orin process (plantain). The plantain is a type of banana, usuallycooked before eating, known as *Musa paradisiaca*. In Indonesia, there are many bananas spesies of plantain such as kepok, tanduk, nangka,raja andgoroho. Goroho is one of a type of plantain, locally grown crops in North Sulawesi and that are not familiar to people outside North Sulawesi, compared to other varieties of banana. Considering the uniqueness and bioactivity of this banana, it might potentially be one of fruits used as food diversification and functional food. People in North Sulawesi usually consume goroho banana by cooking it and then serve it with vegetable and roasted fish, because it is tend to be tasteless. Meanwhile, based on observation in Minahasa society, goroho banana is recommended to be consumed as a diet by people with diabetes mellitus.

DOI= http://dx.doi.org/10.20902/IJCTR.2018.110832

The literature reports that goroho banana have low sugar content (1.97%), lipid (0.35%) and also are a good source of energy. Its mineral composition was also studied, revealing the presence of many minerals, such as potassium, calcium, ferrous, and magnesium. The highest amount of mineral found in goroho banana wass potassium, whilst calcium is the least[1]. The other studies exhibited that goroho possess to phenolic, flavonoid, tannin, antioxidant and antidiabeticactivity and the bioactive properties of this natural resource[2,3].According to many studies, a type banana of other plantain have been identified as good sources of dietary fiber, resistant starch, mineral, natural antioxidants and antiproliferative activities with bioactive properties[4,5,6,7,8,9].

Banana had dietary fiber, phytonutrient and many bioactivities can be processed into flour and used as a composite in some flour products to enhance phytochemicals, diet fiber, mineral, vitamin and macronutrients. According to Noorfarahzilahetetal[10] composite flour is a mixture of several flours obtained from root, tuber, cereal and legume, with or without the addition of wheat our, which is created to satisfy specific functional characteristics and nutrient composition. It is also agreed with that as the composite flours used were either binary or ternary mixtures of flours from some other crops with or without wheat flour[11]. The use of composite flour from various plants can give variety of food product and elevate the phytochemical content, fiber, mineral element, and antioxidant potential that are beneficial for health. Meanwhile, the use of composite flour could decrease the use of wheat.

This is studies,banana composite flour producedby mixture of corn and sago flour. The addition of corn and sago flour, which are rich in macronutriens, micronutrients, dietary fibre, phytochemicals, and bioactivity to composite products in the development of functional foods open up new potential for banana composite. It is also able to improve their nutritional value, appearance, aroma, taste, texture, and diversity of phytochemicals in composite flour. Therefore, processing of raw material can be from some flour mixture from different commodity and to formulated by a particular composition and would be produced composite flour with physicochemical characteristic properties that similary with wheath flour.

Antioxidant phytochemicals presentin composites flour are complex and many properties of plant food are associated with content of their polyphenolic compounds. The food phenolic in composite flour can occur to interaction with charbohydrate, protein and other component. Phenolic found in foods generaly belong to phenolic acid, flavonoid, lignin, stilbenes, coumarines and tannins[12]. Therefore, the solvent choice for the extraction of antioxidants is most important for extraction technique of food phenolics. Many of the food phenolics are soluble in water or organic solvents[13]. Some studies reported that solvents be often used for extraction of food phenolics was methanol, ethanol, acetone, ethyl acetate dancombination of solvent mixture[13,14,15,16]. It is generally with addition of co-solvent as water, helping in extracting of the all class of phenolic compounds from plant tissuess. The objectives of this research were to evaluate phytochemicalcontent and to determine antioxidant activity in composite flour from banana, corn and sago.

Experimental

Standard and Chemicals

The samples were banana, corn and sago taken from farmer land and local market in North Sulawesi. The chemicals were ethanol, methanol, acetone, sodium carbonate, Folin-Ciocalteu reagent, sodium hydroxide, sodium acetate, acetic acid, chloride acid, iron(III) chloride, iron(II) sulfate were pro analysis grade and were sourced from Merck (Darmstadt, Germany). The 2.4.6-tri-pyridyl-s-triazine (TPTZ) was taken from Fluka (Deisenhoten, Switzerland). Gallic acid, β-carotene, and 1.1-diphenyl-2-picrylhydrazyl (DPPH) were sourced from Sigma Chemical Co. (St. Louis, Missouri, USA).

Sample preparation

Goroho banana, baruk sago and corn flour production

Goroho banana was steamed for 15 minutes. After that, the banana was peeled then sliced with a thickness of 2 mm using a stainless steel knife. Banana chips were dried in an oven at 50^{o}C for 9 hours. Dried banana chips then milled using a hammer mill and sieved using 80 mesh sieve. Goroho banana flour was placed in a plastic bag before characterized.Baruk sago stem was split into two pieces then it pith was cut to 1 cm in size. Two hundreds grams of baruk sago pith was extracted with 1000 mL aquades then blended for 5 minutes.

Chiffon was used to filter the mixture. The filtrate was precipitated for one night. The precipitate then dried in an oven at 50°C for 9 hours. Crude baruk sago flour was milled and sieved using 80 mesh sieve. Baruk sago flour was placed in a plastic bag before characterized.Corn kernels were sorted from contaminations. After that corn kernels were washed and dried in and oven at 50°C until dry. The dried kernels were milled to the size that passess80 mesh sieve. Corn flour was placed in a plastic bag before characterized.

Composite flour formulation

Composite flour was produced using mixture of banana, corn and sago flour at various different level (90:0:10, 80:10:10, 70:20:10, 60:30:10 and 50:40:10). Banana, corn, sago mixed as with formula composition by using mixer food processor for 5 minutes. The composite flour was kept in plastic bags at 5 °C prior phytochemical analyzed and antioxidant activity.

Phytochemical extraction

Ten grams of sago starch put into a 250 mL Erlenmeyer flask which was lined with aluminum foil to avoid light, then extracted for 24 hours with 50 mL of methanol-water (80:20, v/v), ethanol-water (80:20, v/v) and acetone-water (80:20, v/v). After that, filtered and the filtrate is evaporated to obtain the composite flour extracts. Subsequently, the 80% ethanol was then added so the volume of the supernatant was 50 mL in exact. The extract stored at 5°C for the preparation of phytochemical analysis and testing of antioxidant activity.

UV spectra

Extraction of phenolic compounds by different solvent system was monitored by means of UV absorption at 200-400 nm using spectrophotometer (Shimadzu 1800). The UV spectra of solvent extraction in methanol, ethanol and aceton were also measured.

Determination of total phenolic content

The total phenolic content of the composite flour were determined using modified Folin-Ciocalteu colorimetric method from Li et al[17]. Each sample solution (0.1 mL, 1 mg/mL)was added to Folin-Ciocalteu reagent (0.1 mL, 50%) in a test tube and then this mixture was vortexed for 3 minutes. After intervals of 3 minutes, 2 mL of Na_2CO_3 2% solution was added. After incubation at room temperature for 30 min, the mixture was kept in the dark for 30 minutes. The supernatant was measured using a spectrophotometer at 760 nm. The standard curve was prepared using different concentrations of gallic acid and the results were expressed as gallic acid equivalents in milligrams per milligram extract.

Determination of total carotenoid

The content of β-carotene in composite flour was determined according to Gross[18]. The sample was dissolved in petroleum ether and measured its absorbancy using spectrophotometer at wavelength of 450 nm. The concentration of carotenes expressed as β-carotene (μg/g) was calculated using the equation as follows:

Equation :

$$\textbf{β-carotene} = \frac{A \times V \times D}{\epsilon \times W} \quad (1)$$

Where:
A: absorbancy, d: dilution, ε: coefficient of absorbancy (2592 for petroleum-ether) w: weight of sample (g), V: volume (mL).

Determination of free radical scavenging DPPH

Determination of free radical activity (scavenger) from sago baruk starch extracts measured by the method Li et al[19] slightly modified. A total of 2 mL solution of 1.1-diphenyl-2-picrylhydrazyl (DPPH) 92 μM in ethanol was added 0.5 mL composite flour extract. The level of colour reduction of the solution shows the efficiency of radical scavenger. The last five minutes of the 30 minutes, the absorbance was measured with a spectrophotometer at 517 nm. Free radical scavenger activity is calculated as a percentage reduction of DPPH color.

Equation :

Free radicals scavenging activity (%) = 100 x$\frac{1-\text{absorbance of sample}}{absorbance\ of\ control}$ .... (2)

Determination of total antioxidant with FRAP method

Determination of total antioxidant determined by methods ferric reducing ability of plasma (FRAP)[20]. Measurement is carried out by taking 0.1 mL pith extract dissolved in ethanol is mixed with 3 mL reagent FRAP in a fresh state. Then the mixture is shaken with a vortex instrument and thereafter, immediately was measured absorbance at 593 nm wavelength. FRAP reagent was always prepared in a fresh state by mixing 2.5 mL, 10 mM solution of 2,4,6-tripiridil-s-triazine (TPTZ) in 40 mMHClmM with 2.5 mL, 20 mL solution of $FeCl_3$ $6H_2O$ and 2.5 mL, 0.3 M acetate buffer at pH 3.6. The total content expressed as equivalent antioxidant Fe^{3+} to Fe^{2+} in mol/L extract. A standard curve was prepared in the same way using $FeSO_4$ solution with a concentration of between 100-1000 mol L^{-1}.

Statistical analysis

All the experimental data do triplicates and the results are expressed as mean ± SD. Analyzes were performed using SPSS software version 18.

Result and Discussions

Total phenolic content

Total phenolic content of composite flour was determined by using Folin-Ciocalteu reagent. The mechanism is based on the reduction of phosphomolibdate-phosphotungstate complex in Folin-Ciocalteu reagent by phenolic compound contained in sample. The complex is then turned into molybdenum, which can be identified qualitatively by the formation of blue color and thus detectable with a spectrophotometer at 750 nm. According to Huang et al[21], all phenolic substances including simple phenols can react with Folin-Ciocalteu reagent.

Table 1. Phytochemicals content

Sample	Solvent	Total Phenolics			Total Carotenoids		
F1	Methanol	46.90^{abk}	±	2.95	5.93^{ak}	±	0.28
	Ethanol	47.04^{bk}	±	2.83	9.62^{bk}	±	0.42
	Acetone	35.20^{ak}	±	5.55	8.39^{ck}	±	0.35
F2	Methanol	53.98^{abl}	±	3.36	9.70^{al}	±	0.17
	Ethanol	50.85^{bl}	±	1.31	8.03^{bl}	±	0.48
	Acetone	40.10^{al}	±	1.24	9.70^{cl}	±	0.64
F3	Methanol	59.90^{abm}	±	6.01	8.54^{am}	±	0.29
	Ethanol	59.63^{bm}	±	0.12	12.23^{bm}	±	0.55
	Acetone	63.37^{am}	±	1.24	12.88^{cm}	±	0.54
F4	Methanol	61.73^{abn}	±	5.23	9.91^{an}	±	0.17
	Ethanol	70.65^{bn}	±	1.83	12.09^{bn}	±	0.49
	Acetone	72.55^{an}	±	3.36	14.62^{cn}	±	0.59
F5	Methanol	61.19^{abm}	±	6.30	8.83^{ao}	±	0.39
	Ethanol	65.88^{bm}	±	0.77	11.87^{bo}	±	0.71
	Acetone	65.82^{am}	±	1.95	17.52^{co}	±	0.80

Data are expressed as the mean ± standard deviation (N=2). Means with different letters are significantly different (p<0,05).

Statistical analysis revealed that total phenolics of samples with different solvent were significantly different (p<0,05). However, total phenolics of samples which extracted with acetone and ethanol, and methanol and ethanol did not show any differences (p>0,05). Based on the result presented in Table 1, composite flour extracted with methanol and ethanol show higher concentrations of total phenols compared to acetone extract in F1. This is due to the high polarity of methanol and ethanol, whilst acetone is less polar. In F2, the highest concentration of total phenols was measured in methanol extract, while the ethanol extract possessed the lower total phenols yet higher than acetone extract. These results were influenced by the polarity of solvent, because methanol was the most polar solvent used in this experiment, while ethanol and acetone in descending order were less polar than methanol. Meanwhile, acetone extracts in F3, F4, and F5 possessed the highest phenol content, which then proved that high solubility of phenol does not always depend on the polarity of solvent, but it is also based on the structure of phenol. Because phenolic components (phenolic acid, flavonoid and tannin) are easily dissolved in polar solvents, the solubility of phenolic in ethanol is higher than methanol. Meanwhile, acetone is a dipolar-aprotic solvent, which possess a large dipole moment and donor properties, have no acidic proton, while methanol and ethanol are a polar protic solvent, which possess a proton-donating fungtion and a large dipole moment[22]. It can easily interact with polar functional group in phenolic through capacity for hydrogen bonding.Based on the result of the five extracts, total phenolic contents were affected by the solvent and formulation used in the extracts.

Total carotenoid content

The carotenoid total content of different solvent extracts from the five formulas are shown in Figure 3. Statistical analysis revealed significant differences among the total carotenoid content of all composite flours (p<0,05). All formula showed that extraction solvent for the methanol-water (80:20, v/v) extract had the carotenoid content ranged from 5.93 to 9.91µg/mL and for the 80% ethanol extract ranged from 8.03-12.23 µg/mL, whereas acetone extract ranged 8.39-17.52 µg/mL. These results indicated that extraction solvent had a different significant (p<0.05) influence on carotenoid content.Among five formula extracts analyzed, 80% acetone extract exhibited the highest carotenoid content, followed by ethanol (80:20, v/v), methanol (80:20, v/v), respectively. These results found that 80% acetone potentially enhanced the carotenoid extraction yield followed by 80% ethanol and 80% methanol. Carotenoids are soluble in apolar solvents, including edible fats and oils. Because carotenoids are liposoluble, they are usually extracted from the plant sources with organic solvents such as chloroform, hexane, acetone, and petroleum ether[23]. The samples can contain large amounts of water; water-miscible organic solvents such as ethanol are also used. One of the problems is the elimination of the residual solvents to obtain a safe extracts, this can be avoided by using food grade solvents such as ethanol[24]. Therefore, to obtain the carotenoid concentrate of food plant in semi-manufacturing conditions, ethanol was used as solvent.

The addition of corn flour atconcentration of 10, 20, 30 and 40% showed increasing carotenoid content in each formulae of composite flour especially acetone and ethanol solvent. For ethanol and methanol solvent of formulae have lower carotenoid content compared by ecetone. The acetone solvent can dissolve more carotenoid compound than ethanol and methanol solvent. These results indicated that carotenoid compound found in formulations having semipolar properties. According Hu and Xu[25]repoted that corn containing carotenoid compounds such as lutein and zeaxantin.There is hydroxyl groups in zeaxantincomponent show this compound more polar comparison with its other analogue like α-carotenoid dan β-carotenoid. Therefore, acetone was chosen as the extraction technique in laboratory conditions.

UV spectra

Figure 1 showed the absorption spectra of acetone, ethanolic and methanol extracts measured in ethanol with the wavelength regions of 200-400 nm for phenolic component and 400-600 nm for carotenoid component. Characteristic phenolic and carotenoid component in three solvents of composite flour formula were identified by UV spectrometry. Therefore, the variation components of composite flour formula were extracted in three different solvents such as methanol, ethanol and acetone, indicating the polar and semi polar in the composite flour extract. As shown in Figure 5, the UV spectra of composite flour extract was greatly dependent on the polarity of the extracting solvent. The acetone solvent exhibited maximum (200-400 nm) absorbance were at 324 and 282 nm respectively, whereas ethanol (279 and 324 nm) and methanol (322 and 282 nm).The acetone solvent exhibited maximum (400-600) absorbance were at 478 and 450 nm respectively, whereas ethanol (450 and 477 nm) and methanol (450 and 478 nm).

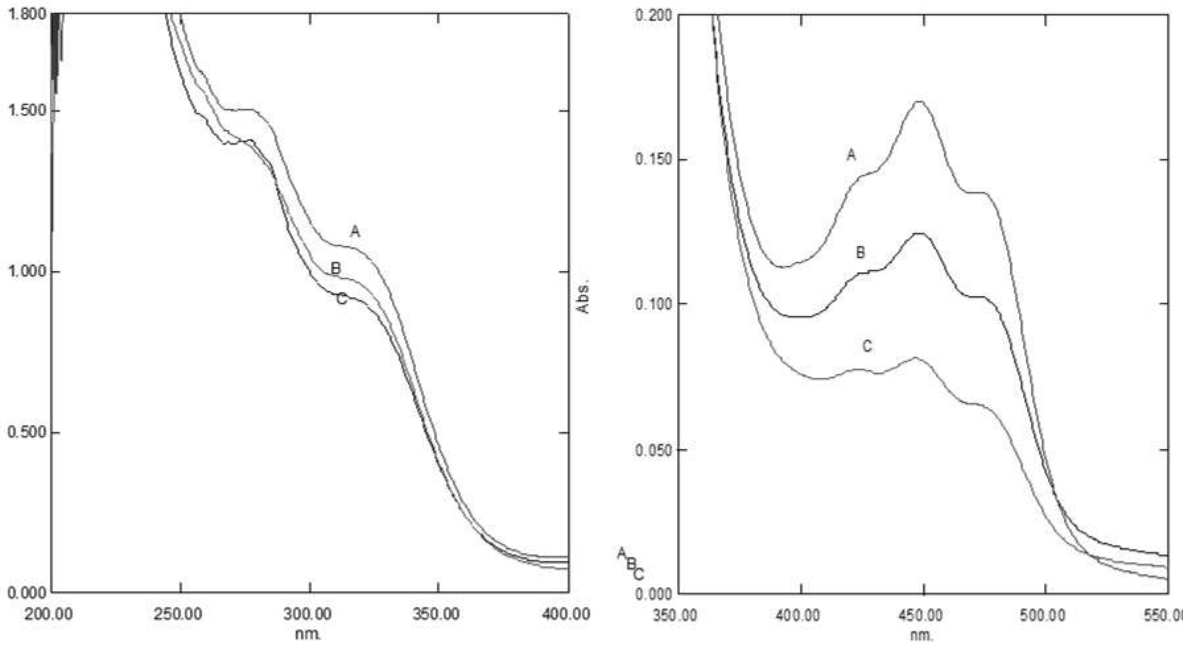

Fig. 1: Absorbance spectral of 2.5 mg/mL acetone (A), ethanol (B) and methanol (C) solvent

These result proved that UV spectra of the acetone, ethanol and methanol extract showed ability for extracting phenolics and carotenoid components. In addition to the UV spectra of acetone solvent was a more effective solvent for obtaining of phenolic and carotenoid component of composite flour formula sample. This could be due to the presence of large amounts phenolic and carotenoid compound, especially flavonoid group, lutein, zeaxanthine and β-carotenoid group that are more soluble in acetone than ethanol and methanol. From Figure 5 also found that UV spectra of formula F5 having characteristic absorption bands in regions 200-400 nm and UVB and capable play role as the photo-protective effect. Sayre et al[26] stated that 400-600 nm ability of active component in composite flour extract associates with antioxidant activity. These results indicate that acetone solvent which to contain phytochemicals such as flavonoid and carotenoid compounds and potential as antioxidant active component.

Free radical scavenging activity

Determination of antioxidant activity on banana flour, corn flour, and sago flour formulas using DPPH (*1,1-diphenyl-2-picrylhydrazyl*) radicals as testing media. Antioxidant method using DPPH was chosen because this method is simple and easy to evaluate antioxidant activity from natural product[27]. This method was done by mixing an extract solution with DPPH then the absorbance of solution was read using spectrophotometer at 517 nm. Radical scavenging mechanism in this method is hydrogen donation mechanism from antioxidant compound, so this free radical receive one electron from antioxidant[28].The compound that acts as free radical scavenger will reduce DPPH which can be seen from DPPH color changing from violet to yellow, when an electron from DPPH radical already paired with hydrogen from a free radical scavenging compound to form reducted DPPH-H[29]. Therefor, the faster the declining of absorbance, the more potential of an extract as an antioxidant.

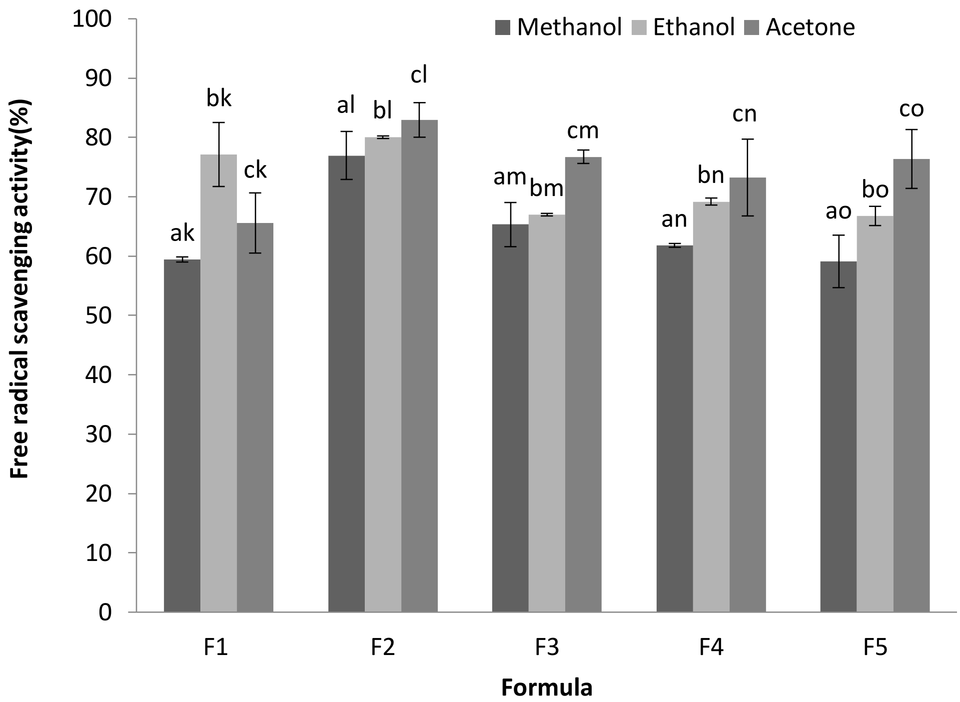

Fig. 2: Radical scavenging activities of three solvents in composite flour formula. Data are expressed as the mean ± standard deviation (N=2). Means with different letters are significantly different (p<0,05).

Figure 2. shows free radical scavenging activity of mixed goroho banana, corn and sagoflour formulations, which extracted using methanol, ethanol and aceton solvent, repectively. Statistical analysis (one-way ANOVA) revealed significant differences among the free radicals scavenging activity of all composite flours (p<0,05). It also revealed that type of sample and solvent were influenced to free radical scavenging activity. The result shows that in formulation I, antioxidant compound were extracted in ethanol solvent. This is caused by antioxidant compounds easily disolved in organic solvent. DPPH radicals react with antioxidant compounds in goroho banana and sago flour formulation by hydrogen donation mechanism[28]. Formulation II-V shows that antioxidant compounds more extracted in aceton solvent compared with methanol and ethanol. Aceton is a semi polar solvent that can extract more phenolic from the formulation. Thus, semi polar compounds extracted with aceton posess better antioxidant activity compared with ethanol and methanol. Formulation II exhibit the best antioxidant activity.

Total antioxidant capacity

Determination of total antioxidant content of various formulations in this study was conducted using FRAP (Ferric Reducing Antioxidant Power) method. The FRAP method works based on the reduction of the ferroin, Fe^{3+} complex of tripeididyltriazine Fe $(TPTZ)^{3+}$ into Fe^{2+}, Fe $(TPTZ)^{2+}$ complexes that are blue by antioxidants in acidic conditions. Total antioxidant content in formulations are interpreted by the increasing of absorbance at wavelength 596 nm, and can be summarized as the amount of Fe^{2+} equivalent to standard antioxidants[30].

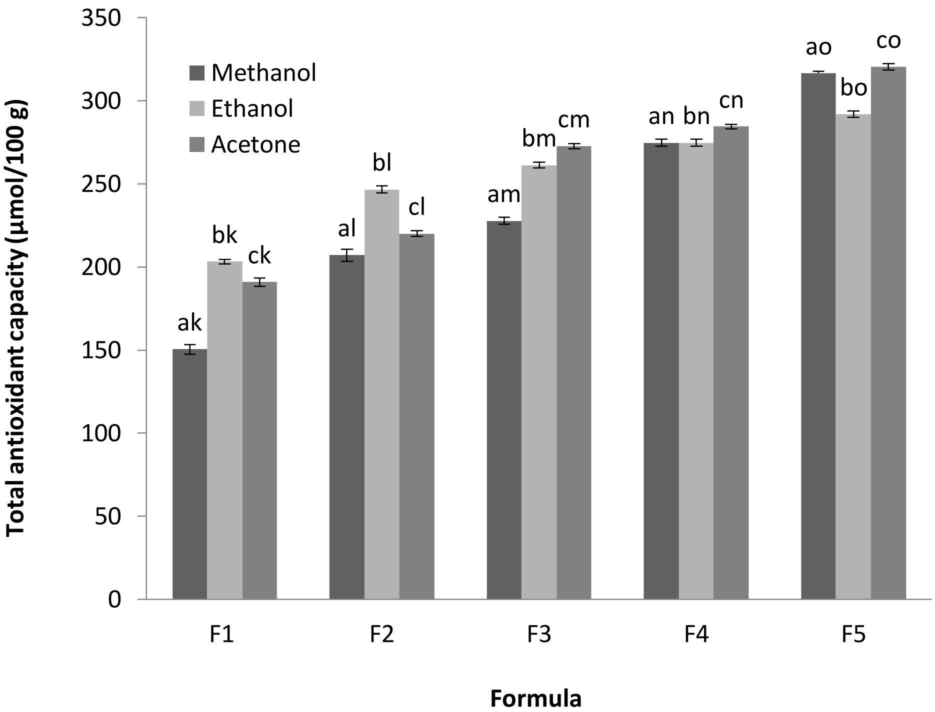

Fig. 3: Total antioxidant capacity of three solvents in composite flour formula. Data are expressed as the mean ± standard deviation (N=2). Means with different letters are significantly different (p<0,05).

Figure 3 shows total antioxidant content of five flour formulations which extracted using methanol, ethanol and acetone. Statistical analysis (one-way ANOVA) revealed significant differences among the total antioxidant capacity of all composite flours (p<0,05). It also revealed that type of sample and solvent were influenced to total antioxidant capacity. First and second formulations which extracted with ethanol shows high total antioxidant content compared to methanol and acetone. However, the highest total antioxidant content in formulations III-V is the formulation which extracted using methanol than ethanol and acetone. The higher total antioxidant content in formulations means more compounds can reduce Fe^{3+} to Fe^{2+} (blue), and compounds that reduce Fe^{3+} is an antioxidant compound. Prior et al[31] states that methanol is a polar compound which is easy to position the hydrogen atoms of a compound or hydroxyl groups to form hydrogen bonds because the bonds would facilitate the transfer of protons (hydrogen atoms antioxidants).

The result shows that antioxidant activity index of extract with polar solvent can be classified as high antioxidant compared to ethanol and acetone extract. Dielectric constant of methanol is higher than ethanol and acetone. Methanol extract can be developed as a component of functional foods or neutraceutical which possess antioxidant activity. The best formulation found in F5.Based on the results of this study, it can be concluded that phytochemical screening on the test (phenolicand tannin), the highest total phenolic content and total tannin content are found in Formulation 2 (F2), while the highest total flavonoid content in formulation 5 (F5). For the highest total carotenoid content is found in Formulation 2 (F2). The highest antioxidant activity (free radical DPPH scavenging activity, and reducing ability) are found in Formulation 2 (F2), although the highest total antioxidant found in Formulation 5 (F5).

Conclusion

Results showed that extraction co-solvent mixtures had significant influence on phytochemicals content and antioxidant activity. The highest total phenolic content was found in 80% methanol and 80% ethanol for F4 and F5, whereas F3, F2 and F3 were found in 80% acetone in composite flour. Besides that, acetone 80% in F5 showed the highest total carotenoid content followed with F4, F3, F2 and F1. Conversely, 80% of acetone in F2 showed the highest free radical scavenging activity comparable that of F1 F3, F4 and F5. The results also showed that 80% of acetone in F3has the highest total antioxidant capacity than F4, F3, F2 and F1. The results suggest that 80% of acetone to extract phenolic phytochemicals in composite flour from banana, corn and sago.

Conflict of interest statement

We declare that we have no conflict of interest.

References

1. Suryanto, E. Antioksidandalampisanggoroho: FitokimiadanKesehatan. Putra Media Grafindo, Bandung, 2015.
2. Suryanto E, Momuat LI, Taroreh M, Wehantouw F. The Potency of antioxidant polyphenol from goroho banana (*Musa sapien*Sp.). AGRITECH. 2011. 31: 289-296.
3. Kaempe HS, Suryanto E, Kawengian SES. Potency of goroho banana extract (*Musa* spp.) on blood glucose of white rats (*Rattusnorvegicus*). Chemistry Progress. 2013. 6: 6-9.
4. Kayisu K, Hood LF, Vansoest PJ. Characterization of starch and fiber of banana fruit. Journal of Food Science. 1981. 46: 1885-1890.
5. Tribess TB, Herna´ndez-Uribe JP, Me´ndez-Montealvo MGC, Menezes EW, Bello-Perez LA, Tadini CC. Thermal properties and resistant starch content of green banana flour (Musa cavendishii) produced at different drying conditions. LWT-Food Science and Technology. 2009. 42(5), 1022-1025
6. Prior RL, Cao G. Antioxidant Phytochemicals in Fruits and Vegetables: Diet and Health Implications. Horticultural Science. 2000. 35: 588-592.
7. Luximon-Ramma A, Bahorun T, Crozier A. Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. Journal of The Science of Food and Agricultural. 2003. 83: 496-502.
8. Sun J, Chu YF, Wu X, Liu RH. Antioxidant and antiproliferative activities of common fruits. Journal of Agricultural and Food Chemistry. 2002. 50: 7449-7454.
9. Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry. 1996. 44: 701-705.
10. Noorfarahzilah M, Lee JS, Sharifudin MS, MohdFadzelly AB, Hasmadi M. Int Food Res J., 2014, 21: 206 1-2074.
11. Shittu T, Raji AO, Sanni LO. Bread from composite cassava-wheat flour: I. Effect of baking time and temperature on some physical properties of bread loaf. Food Res Int. 2007. 40: 280-290.
12. Dey PM,Harbone JB. Methods in plant biochemistry: Plant phenolics.1989.Academic Press, London
13. Zhao ZH, Egashira Y, Sanada H. Phenolic antioxidants richly contained in corn bran are slightly bioavailable in rats. Journal of Agricultural and Food Chemistry. 2006.53: 5030-5035.
14. Druzynska B, Stepniewska A, Wołosiak R. The influence of time and type of solvent on efficiency of the extraction of polyphenols from green tea and antioxidant properties obtained extracts.ActaScientiarumPolonorumTechnologiaAlimentaria. 2007. 6: 27-36.
15. Alothman M, Bhat R, Karim AA. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry. 2009.115 : 785-788. DOI :10.1016/j.foodchem.2008.12.005.
16. Jaiswal AK, Rajauria G, Abu-Ghannam N, Gupta S. Effect of different solvent on polyphenolic content, antioxidant capacity and antibacterial activity of Irish York cabbage. Journal of Food Biochemistry. 2011.36: 1-15.
17. Li XC, Lin J, Gao Y, Han W, Chen D. Antioxidant ability and mechanism of *RhizomaAtractylodesmacrocephala*. Molecules. 2012.17: 13457-13472. doi:10.3390/molecules171113457.
18. Gross J. Pigment in vegetables, chlorophylls and carotenoids. 1991.Van Nostrand Reinhold, USA.
19. Li XC, Wu XT, Huang L. Correlation between antioxidant activities and phenolic contents of radix *AngelicaeSinensis*(Danggui). Molecules. 2009. 14:5349-5361. doi: 10.3390/molecules14125349.
20. Szydlowska-Czerniak A, Dianoczki C, Recseg K, Karlovits G, Szlyk E. Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. Talanta 2008. 76: 899-905 https://doi.org/10.1016/j.talanta.2008.04.055.
21. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry. 2005. 53: 1841-1856. DOI: 10.1021/jf030723c.
22. Isaacs NS. Physical Organic Chemistry. 1987.Longman Scientific & Technical, Longman Group UK Ltd., England.
23. Belitz H.D, Grosch W, Schieberle P. Food Chemistry. 2004.Springer Verlag, Berlin.

24. Calvo MM, Dado D, Santa-Maria G. Influence of extraction with ethanol or ethyl acetate on the yield of lycopene, β-carotene, phytoene and phytofluene from tomat peel powder. European Food Research and Technology. 2007. 224: 567-571. DOIhttps://doi.org/10.1007/s00217-006-0335-8.
25. Hu Q-P,Xu JG. Profiles of carotenoids, anthocyanins, phenolics, and antioxidant activity of selected color waxy corn grains during maturation. Journal of Agricultural and Food Chemistry. 2011. 59: 2026-2033. doi: 10.1021/jf104149q.
26. Sayre RM, Agin PP, Levee GJ, Marlowe E. A Comparison of in vivo and in vitro testing of sunscreening formulas. Photochemistry and Photobiology. 1979. 29(3), 559-566.DOI: 10.1111/j.1751-1097.1979.tb07090.x.
27. Fagliano V. Method for Measuring antioxidant activity and its application to monitoring the antioxidant capacity of wine. Journal of Agricultural and Food Chemistry. 1999. 47: 1035-1040.DOI: 10.1021/jf980496s.
28. Pokorny J, Yanishlieva N, Gordon MH. Antioxidant in food: Practical Application. 2001.CRC Press, Boca Raton.
29. Molyneux P. The Use of stable free radical Diphenylpicrylhyrazyl (DPPH) for estimating Antioxidant activity. SongklanakarinJournal of Science Technology. 2004. 26: 211-219.
30. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods For testing antioxidant activity. Analyst. 2002. 127: 183-198. http://dx.doi.org/10.1039/B009171P.
31. Prior RL, Wu X, Schaich K. Standardized Methods for The Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. Journal of Agricultural and Food Chemistry. 2005. 53: 4290-4302. DOI:10.1021/jf0502698.

*****